Volume 30, Number 2, November, 1957

The Yale Journal of Biology & Medicine

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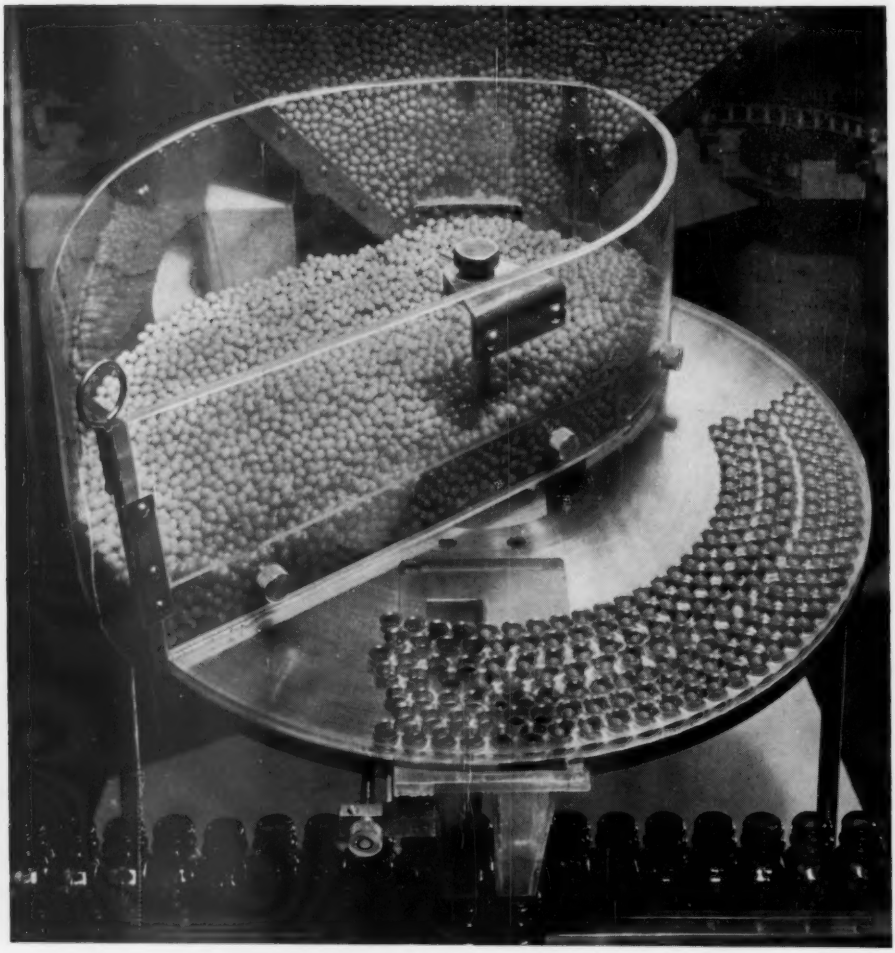

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THE YALE JOURNAL
OF BIOLOGY AND MEDICINE
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Subscriptions. THE YALE JOURNAL OF BIOLOGY AND MEDICINE is published in September, November, December, February, April, and June from 333 Cedar Street, New Haven 11, Connecticut, U.S.A. The subscription price for one volume of six issues is $7.50 in the United States and other countries. For students registered in any school of Yale University, the subscription price is $4.00 per volume. Single copies are $1.50 each except for special issues.

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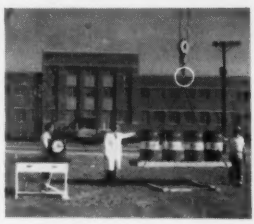

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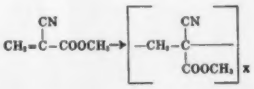

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DOROTHY M. HORSTMANN*

Section of Epidemiology and Preventive Medicine and Department of Pediatrics, Yale University School of Medicine

POLIOMYELITIS: PROBLEMS IN PATHOGENESIS AND IMMUNIZATION**†

The pathogenesis of poliomyelitis has been studied intensively during the past fifty years by many investigators, yet our understanding of the course of events in the human infection is far from perfect. There are still many controversial issues, in general the same issues with which the early investigators were also concerned: viz., is poliomyelitis an entirely neural disease, or is there a systemic, extraneural phase? In what tissues does virus first multiply? How does it invade the central nervous system—from the blood or exclusively by neural pathways? What are the immune barriers against infection and paralysis and where do they operate?

In the discussion today, I should like first to review the current status of some of these problems, and subsequently to describe observations on humans infected with attenuated strains of poliovirus as they relate to certain aspects of the pathogenesis and immune mechanisms.

Current concepts of the nature of poliomyelitis lean toward an interpretation of the infection as primarily an extraneural one in which significant CNS invasion occurs only rarely. This is not a new idea. It was in fact the point of view expressed by Peabody, Draper, and Dochez in 1912[40] and by other investigators at that time. But it was lost sight of in the enthusiasm for neurotropic aspects of the infection which were studied so extensively in the ensuing 25 years, largely by inoculating monkeys either intranasally or directly into nervous tissue and employing almost exclusively the MV strain, which had become highly neurotropic by many neural passages. The return to a more natural perspective in terms of the human infection came in the early 1940's, when through the work of Harmon,[21] Trask, Vignec, and Paul,[49] Sabin and Ward,[46] and others, it became apparent that infection of the alimentary tract is an important phase in the pathogenesis of poliomyelitis. Renewed interest in the pathogenesis of poliomyelitis was stimulated by the discovery beginning in 1951 that a phase of viremia occurs in

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* Associate Professor of Preventive Medicine and Pediatrics.

** Eighth Annual Don W. Gudakunst Memorial Lecture, presented at the School of Public Health, University of Michigan, on March 12, 1957.

† Work reported from the Yale Poliomyelitis Study Unit aided by a grant from the National Foundation for Infantile Paralysis.

Received for publication May 22, 1957.

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orally infected monkeys and chimpanzees[1, 28] and also in the natural human infection.[5, 28] Subsequently, the use of quantitative tissue culture methods of virus and antibody assay and the many new observations by Koprowski *et al.*[29–31] and Sabin[41, 42, 44] on humans infected with attenuated strains have considerably enlarged our understanding of the pathogenesis of poliomyelitis. There is now general agreement on the alimentary portal of entry, and it is known that the vulnerability of different parts of the alimentary tract to virus implantation and multiplication differs in different species. In man, the lower intestinal tract (ileum) is more susceptible than the throat, while the reverse is true in monkeys[11] and chimpanzees.[41] Sabin[41] has demonstrated that larger doses of virus are required to infect the throat than the intestinal tract of susceptible persons; we have found the same to be true in reinfection of naturally immune individuals.[27] The fact that the throat can be by-passed—as it was in children fed attenuated virus in capsules in a human trial to be described later—adds further evidence that multiplication of poliovirus in the throat is not an essential part of human infection.

SITES OF PRIMARY VIRAL MULTIPLICATION

Does virus multiply first in mucosal cells or in lymphoid tissues of the alimentary tract? Or does it avoid both of these and make its way to regional ganglia before multiplying?

The speed with which virus appears in the throat and feces[27, 41, 42] in humans after ingestion of attenuated strains—as short as 24 hours—would seem to narrow the field to a readily available cell, either lymphoid or epithelial. Although Faber *et al.*[13] have shown that virus inoculated directly into ganglia can multiply and be excreted into the throat and intestinal lumen, it would seem unlikely, as Bodian[4] has pointed out, that this cycle of invasion, multiplication, and excretion could occur as rapidly as within 24 hours.

The available evidence favors a primary extraneural phase of viral multiplication in the intestinal tract, but whether in epithelial mucosal cells or those of the lymphoid structures or both is not clear at the present time. The experimental evidence on early infection of the alimentary tract is meager, being based largely on a few chimpanzees sacrificed at various intervals after virus feeding.[8, 42] It is clear, however, that the sites with the largest amounts of virus, presumably due to local multiplication, are the throat and the ileum, both being areas rich in lymphatic structures. Tonsils and Peyer's patches dissected away from pharyngeal or intestinal wall have been assayed for virus, and in a few instances the washed wall of these areas has also been tested. High titers have been found in the tonsils by Bodian,[8]

but washed pharyngeal wall was not tested; Sabin,[49] on the other hand, found high titers in the pharyngeal wall but did not test the tonsils. It is difficult, therefore, to compare these two sets of results. Another problem is that the complete separation of the two tissues of the alimentary tract is virually impossible. There are many submucosal collections of lymphoid cells not necessarily as large as follicles distributed widely in the intestinal tract; virus isolated from "washed wall" might be associated with these cells rather than with the epithelial cells. On the other hand it is equally difficult to rule out multiplication in epithelial cells when assaying Peyer's patches and tonsils for virus. This is due to the fact that, as has recently been pointed out to me by Dr. Ernest Goodpasture,[18] both of these structures have a certain anatomical feature peculiar to them among lymphoid tissues. This consists of the presence of deep crypts extending far into the substance of the follicles—deep crypts which are lined with mucosal epithelial cells. In assaying for virus it would therefore be extremely difficult to separate the two types of tissue. One might even speculate that the localization in tonsils and Peyer's patches in the throat and ileum is due to the presence of these epithelial-lined recesses which form the most favorable sites for virus to lodge, adsorb, penetrate cells both epithelial and lymphoid and begin multiplying. In any event, here as elsewhere in the alimentary tract, in order to reach lymphoid cells, virus must penetrate through—or between—mucosal cells, another reason to suspect that both tissues might support virus growth.

The absence of lesions in either intestinal lymphoid or epithelial cells of orally infected monkeys and chimpanzees has been regarded by Faber[30] as strong evidence against their being sites of virus multiplication. This does not seem a valid argument, since it has been demonstrated that poliomyelitis virus[38] as well as others[45, 47] can multiply *in vitro* to high titers in cells without causing detectable alterations in cell morphology. Further, the isolation of virus in considerable amounts from lymph nodes and intestinal wall of chimpanzees[5, 48] indicates that multiplication *in vivo* can proceed without causing visible cell damage. From the point of view of the virus, this property of being able to live happily without disadvantage to the cells of its human host would seem essential to survival.

Although absence of lesions of lymphoid tissues is a feature of the incubation period in the experimental disease, this is not true of fatal human infections, for studies of lymphoid structures at autopsy have demonstrated, in addition to virus,[80] mild to marked histological alterations. Comments on the involvement of the reticulo-endothelial (R.E.) system were frequent in papers on poliomyelitis 25 years ago. Burrows[7] in 1931, on the basis of observations on cases dying early in the course, went so far as to consider

poliomyelitis a disease of the lymphatic system, which occasionally might involve the CNS. Landon and Smith[28] who reported 96 fatal cases in the 1931 New York City epidemic, probably the largest published series of complete human autopsies, noted that "the most exaggerated picture of the lymphatic structural involvement in poliomyelitis was that seen in the lymphoid tissues of the lower ileum and cecum." The changes included congested, hyperplastic follicles and Peyer's patches, and in some instances erosion and ulceration; all of these were much more striking in patients dying early in the course rather than later in convalescence. Similar alterations were found in the tonsils and spleen, but only minimal changes in the peripheral nodes. More recently, in 1951, these findings were corroborated by Sommers *et al.*,[48] who attempted to determine their specificity by examining lymphoid tissues from more than 50 autopsied cases of various acute childhood infections, both viral and bacterial. In only two of these controls were lesions detected comparable to those found in 82 per cent of poliomyelitis cases.

Although observations on fatal human cases do not indicate what happens in the early, primary phases of infection, yet the regular and often extensive involvement of the lymphatic structures, taken together with evidence of the early presence in them of virus in orally infected monkeys[35] and chimpanzees,[8, 48] indicates that they may play an important role in the natural infection.

VIREMIA

Although viremia has been demonstrated to occur with some regularity in natural human infections,[5, 38] there is now extensive evidence that in fully susceptible, antibody-negative individuals, viremia occurs very rarely following ingestion of laboratory attenuated strains.[9, 20, 48] There is also evidence of marked differences even between virulent strains in their capacity to invade the blood stream. Wenner and Komitsuka[51] demonstrated titers as high as 10^5 and 10^7 TCD_{50} per ml of blood in monkeys inoculated intramuscularly with a virulent Brunhilde strain of type I; in orally infected animals, Bodian[3] found the Mahoney strain at levels up to 10^5 TCD_{50} in the serum, while others, less paralytogenic, such as Y-SK (type II) and Saukett (type III) rarely exceeded 10^1 TCD_{50}.

Current problems associated with viremia include the meaning of this relationship between virulence and degree of blood stream invasion, the source of virus in the blood, and its significance in the pathogenesis of the CNS infection. As to the source of virus in the blood, the speed with which viremia can appear after oral infection with virulent strains in monkeys and

chimpanzees (in two or three days or even less) suggests that invasion of the blood stream occurs from sites of early multiplication either in the alimentary tract or other extraneural tissues. Sabin[48] has pointed out that it is not likely to be the alimentary tract, since attenuated strains which multiply well in the gut fail to invade the blood in significant amounts. Wenner and Komitsuka[51] demonstrated that high titers of virus can be found in the blood and in a variety of extraneural tissues (lymph nodes, GI tract, muscle) of cynomolgus monkeys inoculated intramuscularly with a Brunhilde strain of high paralytogenic capacity, and sacrificed early enough so that only trace amounts had appeared in the CNS. In orally infected chimpanzees sacrificed in the viremic period but before CNS invasion, Bodian[8] found virus in considerable amounts in the alimentary tract, the regional and distal lymph nodes, and the brown fat. The evidence suggests that with virulent, paralytogenic strains, multiplication occurs extensively in various extraneural sites and this multiplication results in viremia. With attenuated strains, on the other hand, as Sabin has shown,[48] infection is more restricted, involving chiefly the alimentary tract and regional nodes, only minimal multiplication (if any at all) occurring more distally in the R.E. system, spleen, peripheral nodes, and perhaps other tissues; and under these circumstances virus fails to appear in the blood stream.

A close parallel to this course of events has been demonstrated by Ørskov and Jensen (quoted by Madsen[35]) for an enteric infection of mice, induced by feeding Salmonella typhimurium. When fully virulent bacteria were fed, it was found that the organisms could be isolated first from the lymph follicles of the intestinal tract; this was followed rapidly by their appearance in regional lymph nodes, then in more distal parts of the R.E. system, the spleen, liver, and finally in the blood. In contrast, when mice were fed the same strain of bacteria which had been attenuated by serial passage at high temperatures, multiplication was demonstrated at considerable levels in intestinal lymph follicles, to a much lesser extent than with virulent strains in mesenteric nodes, spleen, and liver, and never was the organism cultured from the blood. Further decrease in virulence led to infections which involved only the intestinal lymph follicles.

A comparison of the findings on virus assay of chimpanzees infected with attenuated strains[48] and virulent strains[8] suggests that in poliomyelitis, as with the enteric infection of mice studied by Ørskov and his colleagues,[35] both attenuated and virulent strains multiply in the same sites but to different extents and under different genetic controls. The loss of "invasiveness" or "virulence" characteristic of attenuated strains is associated with a reduced capacity to multiply in extraneural sites, which in turn is associated with absence of viremia.[48]

MECHANISMS OF CNS INVASION

The significance of viremia in poliomyelitis is a part of the problem of how virus reaches the CNS. Does it invade directly from the blood? Or does it travel from the periphery via nervous pathways and is viremia merely an incidental occurrence? This also remains a controversial issue. Actually, there is a good deal to indicate that both mechanisms may play a role under certain circumstances. That poliovirus can travel and invade by way of nervous tissue once inside nerve cells is a well-established fact and has been thoroughly documented by many workers. But it does not follow that such is the required or even the usual course of events in the natural infection.

In summarizing the evidence favoring invasion from the blood, which seems to me the more likely explanation, the remarkably constant association of viremia and CNS invasiveness stands out strikingly. Although this association does not prove that CNS virus is blood-borne, it is difficult to relegate the correlation to a position of insignificance. Other findings which favor CNS invasion via the blood stream include: (i) the demonstration by German and Trask[17] of the regular occurrence of paralysis in monkeys inoculated cutaneously into a completely denervated limb; (ii) the experiments of Bodian[2] in which large doses of a virulent strain of virus introduced intravascularly resulted in paralysis with a short incubation period comparable to that following intracerebral inoculation; (iii) the fact that low levels of serum antibody produced by vaccination[15] or injection of gamma globulin[19] protect against the paralytic disease, most probably by blocking the viremic phase of infection.

Sabin,[48] who favors the view that invasion occurs by the neural route, believes that this blocking effect occurs not at the vascular level but at the cellular one, and that serum antibody prevents the progress of virus from extraneural sites of multiplication in the alimentary tract and elsewhere to peripheral nerve endings with which the cells are in close connection. Faber,[20] whose view is that primary multiplication takes place in ganglia and CNS invasion occurs from peripheral neural connections, visualizes the antibody present in mucus in the oropharynx as blocking primary neural entry. In both Sabin's and Faber's interpretation, antibody is considered to act at the cellular level rather than at the vascular one. If this is the case, however, one would expect that alimentary tract infection as well as CNS invasion would be prevented by the presence of circulating antibody. Instead, alimentary infection has been shown to occur in the presence of even relatively high titers of serum antibody.[37, 44]

The evidence favoring neural invasion rests largely on the demonstration of lesions and virus in peripheral ganglia early in the course of infection

induced by virus feeding. Faber *et al.* have shown this repeatedly in cynomolgus monkeys fed virulent strains of virus. That it is a somewhat irregular occurrence is suggested by the results of Faber and his coworkers[18] and by Bodian's findings that in only 2 of 8 chimpanzees fed virulent strains could trace amounts be detected in trigeminal and coeliac ganglia.[8] Sabin[48] has recently reported that in a chimpanzee infected with an attenuated strain, small amounts of virus were isolated from superior cervical and thoracic spinal sympathetic ganglia. The interpretation of these observations as indicative that CNS invasion necessarily occurs via neural pathways is not convincing. Actually if, as the results indicate, both virulent and attenuated strains may rapidly infect ganglia, this would argue that such peripheral invasion occurs regularly but it is not an important aspect of virulence. Obviously the data are not extensive enough to permit a final definitive interpretation.

INFECTION IN THE PRESENCE OF ANTIBODY

Studies by Brown and his associates,[6] by Fox *et al.*,[14] Horstmann *et al.*,[24] and others[20, 28] have shown that persons who already have specific antibodies as a result of previous natural infection do not readily become infected when exposed to a case of poliomyelitis or poliomyelitic infection in the family. In contrast, more than 90 per cent of susceptible, antibody-negative individuals become infected under similar circumstances. Recent field investigations have been concerned with the question, is there a qualitative difference in the immunity resulting from natural infection as compared to that which follows vaccination with Salk vaccine or inoculation with gamma globulin? Brown *et al.*[6] showed that gamma-globulin-inoculated children, when exposed to a case of poliomyelitis in their families, became infected and excreted virus as readily as did susceptible children who had not received gamma globulin. Similarly, several field studies carried out since 1954[5, 16, 34] indicate that vaccination with formalinized vaccine also fails to affect the incidence of alimentary infection among vaccinees on exposure to an infected person in the home.

This problem has also been investigated by observing the responses to ingestion of attenuated strains of poliovirus by individuals with naturally acquired and formalinized vaccine-induced antibodies. Koprowski *et al.*[29] found that none of 3 individuals with antibodies to type II naturally acquired showed antibody rises, but 2 of them excreted virus on single occasions 5 and 23 days after ingestion of the TN type II strain. Sabin has compared the responses of 8 "naturally immune" and 8 "vaccine immune" persons following feeding of attenuated strains, and found that low levels of naturally acquired antibody protected against alimentary infection,

whereas prefeeding vaccine-acquired antibody at levels up to 256 failed to inhibit multiplication in the alimentary tract in any of the volunteers tested.

We have been concerned with this problem, also, and have carried out two human trials with attenuated strains generously made available to us by Dr. Sabin. In the first one[87] a type III strain was fed to 5 individuals, 4 of whom became infected. The three whose prefeeding antibody was naturally acquired had brief periods of virus excretion in the feces 6 to 9 days, and

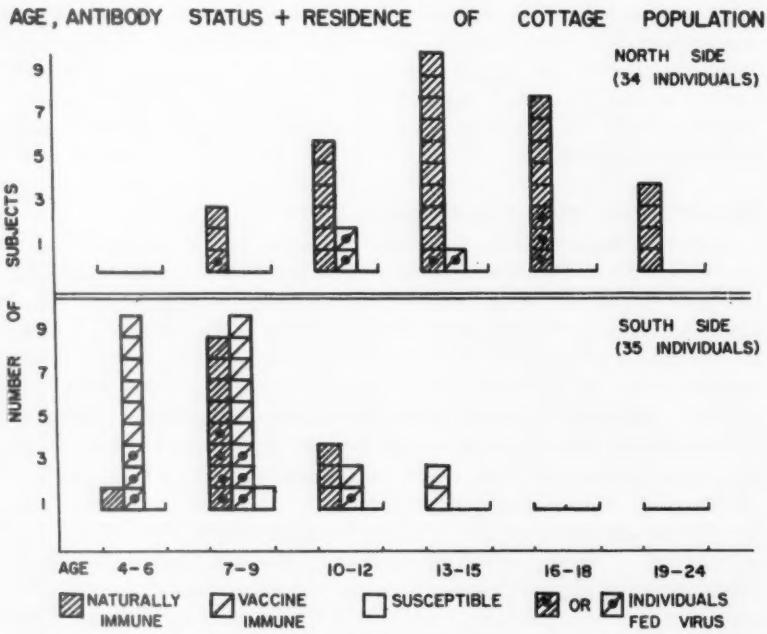

FIG. 1. Age and immune status of 69 persons forming total study population.

prompt neutralizing antibody rises; the fourth individual, a vaccinated child, excreted virus for 6 weeks, and developed CF as well as neutralizing antibody rises. Subsequently we have carried out a trial with an attenuated type I strain, also made available to us by Dr. Sabin who had used it previously to infect adult volunteeers. I should like to describe at this point the results of this second trial, a preliminary report of which has already been given. The observations represent work carried out by the Yale Poliomyelitis Study Unit[88] as a team and form part of a larger program of investigation of pathogenesis and immune mechanisms in poliomyelitis.

HUMAN TRIAL WITH ATTENUATED TYPE I POLIOVIRUS

The trial was carried out in an institution caring for mentally retarded children. The unit used consisted of a cottage housing 69 individuals whose ages ranged from 4 to 24 years. In general, the older individuals resided and had their play area in the north wing, while the younger children were largely in the south wing of the building. All of the children in the cottage either had naturally acquired type I antibodies, or had beeen vaccinated with commercially available Salk vaccine. Twenty were selected for virus feeding, 10 in the vaccine-immune (V) group, and 10 in the naturally immune (N) group—those in each group being matched for age as far as

TABLE 1.

INFECTION FOLLOWING VIRUS INGESTION, CORRELATED WITH NATURE OF IMMUNE STATUS.

RESIDENCE	IMMUNE STATUS			TOTAL INFECTED
	NATURAL	VACCINE	SUSCEPTIBLE	
NORTH	0/5	3/3	—	3/8
SOUTH	2/4	7/7	1/1	10/12
TOTAL	2/9 (20%)	10/10 (100%)	1/1	13/20 (65%)

possible. It turned out later that one child who was thought to have had low-level natural antibody was in fact fully susceptible and reacted with a primary infection.

The strain of virus used was the attenuated L-SC, type I, generously made available to us by Dr. Albert B. Sabin, who had previously used aliquots of the same lot to infect volunteers. The dosage fed to each of 20 test subjects was $10^{7.4}$ TCD_{50}. In an attempt to by-pass the throat, 7 individuals were fed this amount mixed with polyethylene glycol 400 in hard gelatin capsules;* the other 13 were given the same dose in liquid form in milk.

Figure 1 indicates the residence, whether north or south side, the age, and the antibody status of the 20 individuals fed virus, as well as of the rest of the cottage population who formed their close associates. The older indi-

* The capsules and the polyethylene glycol were kindly supplied to us by Dr. Hilary Koprowski, who had used the method previously in feeding virus to children.[81]

viduals living on the north side were chiefly naturally immune; 8 of these (5 N, 3 V) from 8-18 years of age, were fed virus. Of the south side population, 12 (5 N, 7 V, 1 susceptible), aged 4 to 10, were fed.

Of the 20 to whom virus was administered, 13 (65 per cent became infected (Table 1), as evidenced by virus excretion and significant antibody rise. Two of the 9 naturally immune (20 per cent) and all 10 (100 per cent) of the vaccine immunes thus became infected. None of the infected persons developed signs of illness at any time.

The effect of the method of virus administration, whether by capsule or in liquid form, is indicated in Table 2. The results confirm those of Koprowski

TABLE 2.

EFFECT OF CAPSULE OR LIQUID ADMINISTRATION OF VIRUS ON EXCRETION FROM THROAT AND FECES

IMMUNE STATUS	METHOD ADMINISTRATION	VIRUS EXCRETION		TOTAL INFECTED
		THROAT	FECES	
NATURAL	CAPSULE	0/4	1/4	1/4
	LIQUID	0/5	1/5	1/5
VACCINE	CAPSULE	0/3	3/3	3/3
	LIQUID	6/7	7/7	7/7

et al.[21] and indicate that if virus is fed in capsule form, infection of the throat can be by-passed. None of the 7 individuals who ingested $10^{7.4}$ TCD_{50} in capsule form excreted virus in the throat. Four of these were natural immunes, only one of whom became infected as evidenced by fecal excretion. Of the 3 capsule-fed vaccine immunes, none excreted virus in the throat, but all excreted it in the feces. Conversely, positive throat swabs were demonstrated in 6 of 7 vaccinees who were given virus in liquid form. In all in whom the throat was considered infected, throat swabs were negative 6 hours after virus ingestion, but became positive 1 to 3 days later and continued so for a matter of days.

The different patterns of virus excretion and antibody response in the infected individuals is illustrated in Figures 2 through 5. Figure 2 shows the results with subject #553—a 9-year-old boy, prefed naturally

acquired type I antibody at a level of 1 : 64.* Following ingestion of a large dose of attenuated type I virus in capsule form, he became infected, as evidenced by a gradual rise in specific antibody from 64 to 1024 over a period of 56 days. Virus excretion, however, was minimal, positive stool specimens being obtained on the 1st and 6th days only, and the throat being consistently negative. There was no significant CF response. This re-infection, then, in a naturally immune individual resulted in barely detectable amounts of virus in the intestinal tract, yet gave a significant (8 fold) antibody rise.

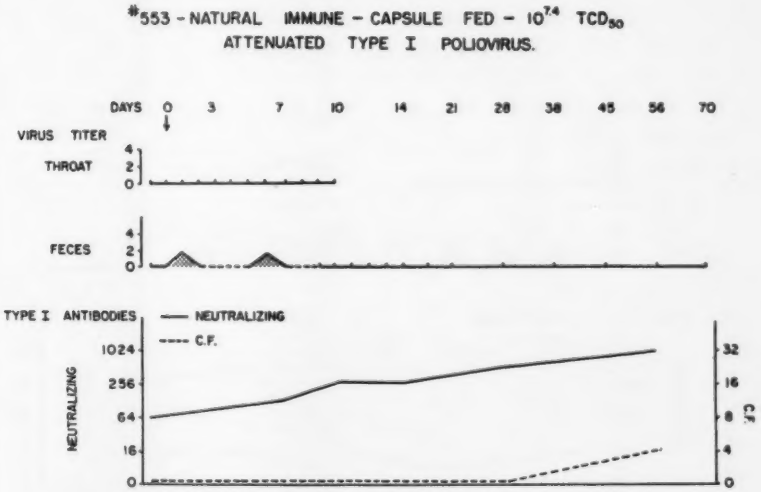

FIG. 2.

Figure 3 is also concerned with a naturally immune person who was fed virus in liquid form. In spite of the large dose, his throat was apparently not infected, and virus excretion in the stools was small in amount and strikingly intermittent. His antibody rise was slow and not very high; there was no significant CF response. The pattern is similar to that in the previous individual who also had naturally acquired antibody, and to the one other naturally immune person who became infected: all of these had minimal and intermittent excretion of virus in the feces, but definite rises in neutralizing antibody.

Turning now to "vaccine-immune" persons who became infected: #503

* The antibody levels of all individuals reported in this study were determined by the colorimetric neutralization test.[88]

(Figure 4) with a prefeeding neutralizing antibody level of 64, was fed virus in liquid form. He excreted virus in the throat from the 1st through the 9th day, in the feces steadily through the 11th day, and intermittently thereafter through the 38th day. He had a sharp rise in both neutralizing and CF antibodies, beginning on the 10th day.

In contrast, with respect to virus in the throat, is #540 (Figure 5), vaccine immune, but capsule fed. With a prefeeding titer of 16, this individual failed to excrete virus in the throat, but excreted it in the stools inter-

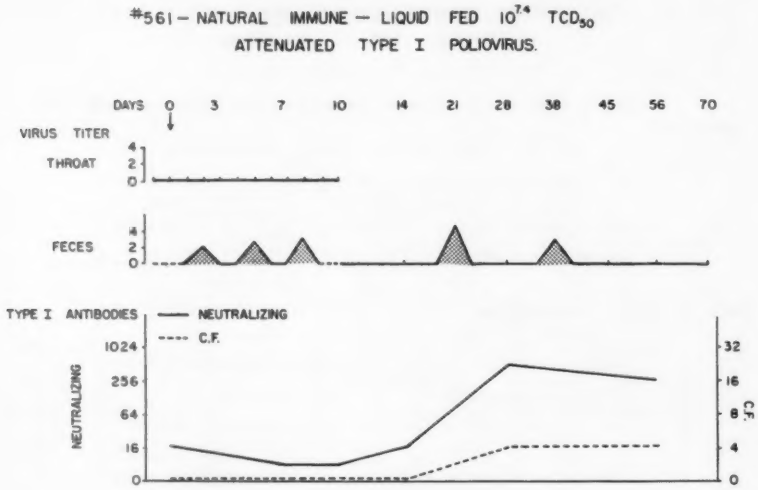

FIG. 3.

mittently from the 3d through the 21st day, in titers, usually of 3.5 to 4.5 logs per gram. Sharp rises in neutralizing and CF antibodies began on days 7 and 10. Thus, although the throat was not infected, this individual excreted virus and developed antibodies as promptly as did those liquid-fed individuals with positive throat swabs.

The patterns of response, then, of naturally immune and vaccinated persons with more or less comparable prefeeding antibody levels who became infected after ingestion of attenuated type I poliovirus, differed, as illustrated in the charts (Figs. 2-5); the 2 vaccinated individuals excreted relatively larger amounts of virus steadily during the first 2 weeks at least, and developed rises in both neutralizing and CF antibodies; the 2 naturally immune excreted little virus in the feces, excretion was intermittent, and

while they developed neutralizing antibody rise, there was no significant CF rise.

Spread of infection to associates. The next problem considered was the ease—or difficulty—with which an attenuated strain of poliovirus might spread to close associates of infected individuals. The population under study consisted of 69 individuals, all living in the same cottage, but roughly divided into two groups, the older in the North Wing and the younger in the South Wing. Essentially the conditions of living were analogous to those

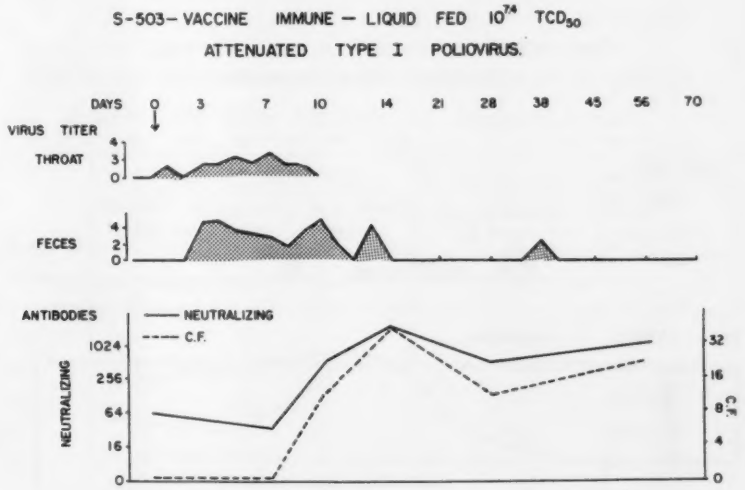

FIG. 4.

existing in family life, but the amount of fecal contamination of the environment was far greater because the subjects were low-grade mental defectives. In this situation, as reported above, 13 of 20 individuals fed virus became infected; and the degree of spread to the 49 associates was considerable (Table 3). Among the naturally immune contacts, 1 of 36 (3 per cent) became infected, while of the vaccine immunes the figure was 12 of 13 or 92 per cent. In all but 2 of the infected contacts, infection was evidenced by virus excretion and antibody rise; in the 2 there was serological evidence of infection but none of the 3 stool specimens collected at intervals of approximately 3, 5, and 7½ weeks after the trial began were positive for virus. These results and these rates of spread are not comparable to those in our previous trial[27] in which the type III strain used did not spread to any

of 7 naturally immune or, one susceptible individual, all of whom were adults, most of them living as ambulatory patients in a hospital ward. The situation is also different from those in which Sabin[42, 44] has used the same type I strain and failed to observe significant spread: his volunteers were all adults of normal mentality.

Incubation period. The speed with which infection with the attenuated type I virus spread to associates of individuals fed in our trial was of interest in terms of the duration of the incubation period in the natural human infection. Although stool specimens from the entire cottage were tested at

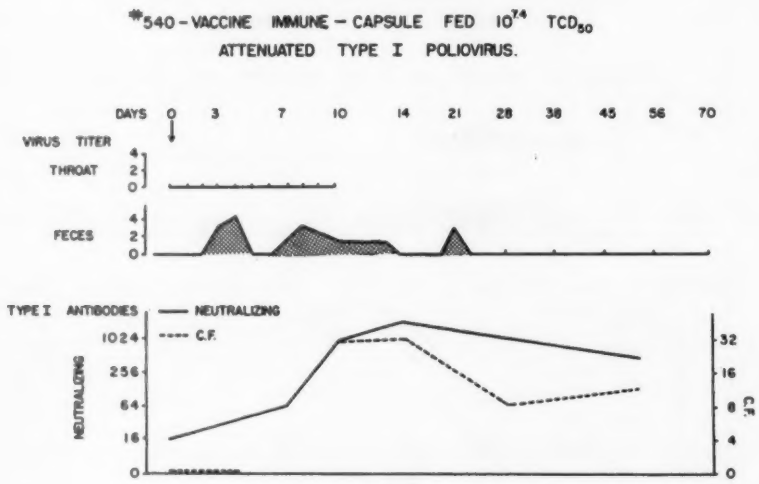

FIG. 5.

intervals, 14 persons (7 V, 7 N) had been selected as controls to be followed by means of more frequent stool and blood specimens. Of the 7 vaccinees in the group, all became infected and in 5 the first stool collected was already positive. This was on the 7th day after the trial had begun, i.e., 7 days after virus had been fed to the 20 test individuals. Thus the incubation period must have been very short—2-3 days in terms of establishment of alimentary infection since in 7 days or less 2 human passages had already occurred. Dick and Dane[9] in a recent report on a similar trial with an attenuated strain of virus collected earlier stool specimens from contacts, one of whom was found to be excreting virus on the 5th day after the test individual had been fed. These observations confirm earlier estimates of

the incubation period by Paul and others based on the occurrence of the "abortive" or minor illness form of poliomyelitis.[38, 39]

Role of antibody level in inhibiting alimentary infection. It was our aim in this human trial to compare infection in naturally immune and vaccine-immune individuals with comparable antibody levels, but because the responses to Salk vaccine were not very high, this was possible in only a limited number of instances. Table 4 correlates the occurrence of infection with the prefeeding type I antibody titers of the 20 individuals fed and their 49 associates, in both vaccine-immune and naturally immune individuals.

TABLE 3.

SPREAD OF INFECTION AMONG 49 ASSOCIATES OF 13 INFECTED PERSONS FED VIRUS. RELATION TO ANTIBODY STATUS.

	SOURCE OF INFECTION	INFECTION OF 49 ASSOCIATES	
	NUMBER OF INDIVID. FED WHO EXCRETED VIRUS IN FECES	IMMUNE STATUS "NATURAL"	"VACCINE"
NORTH	3	0/26	—
SOUTH	10	1/10	12/13
TOTAL	13	1/36 (3%)	12/13 (92%)

Those vaccinees with levels up to 16 all became infected. Unfortunately, there was none among the naturally immune with comparably low levels. Conversely, in the group with levels of 128 or more, there were 29 among the naturally immunes, one of whom became infected, but no comparable group among the vaccinees. Only in the range of pre-exposure levels of 32 to 64 can an adequate comparison be made. Here, 8 of 8 (100 per cent) of vaccinated individuals became infected, but only 2 of 15 (13 per cent) of those who had naturally acquired antibodies. This suggests, as has been pointed out before[27, 41] that a given level of circulating antibodies alone—at least at this 32-64 range—is not the whole story in resistance to infection; something else, conceivably local tissue immunity or conceivably previous high levels of antibody, alter the response.

The question of the stability of the attenuated strain of poliovirus used in this particular trial is being studied by testing the virulence of 1st and 2d

human passage virus isolated from infected individuals late in the course of infection. The results are as yet incomplete.

To summarize at this point the results of our trial with the L-SC attenuated strain of poliovirus in this "closed" cottage population: (i) Of the 69 persons in the group, 27 eventually became infected as evidenced by virus excretion and antibody rises. None of these persons became ill at any time with signs suggestive of poliomyelitis. (ii) Of the 20 test individuals fed virus, 2 of 9 or 20 per cent of the naturally immune and all 10 of the vaccine

TABLE 4.

RELATION BETWEEN ANTIBODY TITER AT TIME OF EXPOSURE AND INFECTION IN 69 INDIVIDUALS

TYPE I ANTIBODY LEVEL-PRE-EXPOSURE	VACCINE IMMUNE		NATURALLY IMMUNE	
	NUMBER	%	NUMBER	%
0-<4	5/5	100	(1/1*)	–
4-16	9/9	100	–	–
32-64	8/8	100	2/15	13
128-256	0/1	–	1/20	5
512-1024	–	–	0/9	0

*SUSCEPTIBLE INDIVIDUAL

immunes became infected. The vaccine-immune group excreted relatively larger amounts of virus for longer periods of time and developed CF as well as neutralizing antibody rises. The two in the naturally immune group excreted minimal amounts of virus intermittently, exhibited slow rises in neutralizing antibody and no significant CF antibody response. (iii) The throat was bypassed successfully by feeding virus in hard gelatin capsules. Of the vaccinees, none of 3 capsule-fed individuals who became infected excreted virus in the throat, while throat swabs were positive in 6 of 7 who were fed the same dose in liquid form. Absence of virus in the throat did not delay its appearance in the stools, nor was there any significant difference in antibody response between throat-positive and throat-negative individuals. (iv) Spread of infection to close associates of individuals fed virus

occurred in 1 of 36 (3 per cent) naturally immune, and 12 of 13 (92 per cent) vaccine-immune contacts. (v) Considering the entire trial, it was possible to compare the ease with which infection was acquired by 15 naturally immune and 8 vaccine-immune persons with comparable type I antibody levels (32 to 64). Two of the naturally immune (13 per cent) and all of the vaccine immunes (100 per cent) became infected.

DISCUSSION

The results of this trial confirm certain earlier observations and provide additional data on human responses to poliomyelitis virus infection, the relative nature of immunity, and the existence of qualitative differences between naturally acquired and vaccine-induced immunity.

As in previous similar studies a difference was observed in these two groups in that individuals with *naturally* acquired antibody were far more resistant to infection than were those with *vaccine*-acquired antibody of approximately the same level. This difference might be explained by assuming that the first line of defense is a tissue barrier (? local immunity) based on one or more previous experiences of the alimentary tract with living, multiplying virus. The second line of defense then becomes the presence of circulating antibody. Another possible explanation is that "tissue immunity" does not enter the picture, but there are qualitative differences in the antibody produced by the two different mechanisms, and higher levels of vaccine-induced than of natural antibody are required to prevent infection of the alimentary tract. There is some evidence that such qualitative differences do exist.[4] Thus Sabin's so-called "low-avidity" neutralizing antibody follows vaccination, and appears also in the acute phase of poliomyelitic infection; in contrast is "high-avidity" antibody which is found in convalescence from natural infection or following infection with an attenuated strain.[21] As to whether very high levels, i.e. >1000, of vaccine-induced antibody will offer the same protection against intestinal infection afforded by lower levels of natural antibody, there is no direct evidence available. However, the experience of Sabin[4] who found that three volunteers with prefeeding titers of 1:256 were readily infected with an attenuated type I strain, indicates at least that moderately high levels of vaccine-induced antibody are not effective in this respect.

Of the many variables involved in determining responses on exposure to polioviruses, the immune barriers and the sites at which they operate are of primary importance in terms of immunization. The present state of knowledge does not allow more than general conclusions about this aspect. It is clear that circulating antibody and probably tissue barriers are involved

in limiting the infectious process, in keeping it localized to the alimentary tract and regional lymphoid structures, in preventing spread to the CNS either from the blood stream, or along neural pathways. The effectiveness of the blocking mechanisms is governed to a considerable extent by the virulence of the infecting strain, and the dosage of virus or degree of exposure. That naturally acquired immunity is more "solid" than vaccine-acquired immunity seems likely, and the probability is great that it is also more durable. Nevertheless, by enhancing the potency of the formalinized vaccine, its effectiveness may, in the future, compare more favorably with that achieved by natural infection. In the meantime, however, there is still much to be learned about human responses to polioviruses. For this reason, we believe that the pursuit of problems of active immunization with attenuated viruses is an important area of investigation in terms of the pathogenesis of poliomyelitis and immunization against the infection as well as the disease.

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STUDIES ON THE PATHOGENESIS OF VASCULAR DISEASE

The Effect of a Fatty Meal on the Course of Acute Inflammatory Lesions of the Coronary Arteries and Aortas of Dogs‡

The present investigation was designed to test the effect of a single high-fat meal on the development and course of the inflammatory arterial lesions in dogs that follow a short, severe episode of hypertension. It was prompted by previous observations that intravenously injected egg-yolk lipids or lipo-proteins from human plasma modify the morphological sequences of certain experimental arterial lesions.[2, 3, 4, 5]

PROCEDURE

Healthy, mongrel dogs of varying ages and weights, previously maintained on low-fat diets, were fed a large, fat-rich meal. The meal consisted of 160 gms. of fresh egg yolk and 230 gms. of canned horse meat. The lipid content of this mixture follows (average of three samples): total cholesterol, .65 per cent, free cholesterol, .38 per cent, fatty acids, 20.3 per cent, lipid phosphorus, 1.6 per cent. Three to five hours after feeding, during alimenary hyperlipemia, the animals' coronary arteries and aortas were injured by a short, intense episode of hypertension induced through repeated intravenous injections of epinephrine. Details of this procedure and a short description of the vascular lesions that follow it have been published.[6, 7, 8] Briefly, the mean femoral arterial pressures of dogs on experiment were maintained over a 30-minute period at near maximal levels (above 200 mm. Hg) by repeated 1 mg. intravenous injections of epinephrine (adrenalin chloride solution 1:1000, Parke-Davis & Co.). Usually a total of from 4 to 8 mgs. of epinephrine was administered. The animals were sacrificed at intervals after the hypertensive episode ranging from a few minutes to three weeks, and appropriate histological examination of their cardiovascular systems and viscera was carried out. In other animals, the effect of the intravenous injection of heparin upon the deposition of lipids in the arterial lesions was investigated. In all, tissues from 39 dogs were examined. Sudan IV stains of frozen sections were utilized for the demonstration of lipids, and a modified Schultz reaction for steroids. Paraffin sections were stained with hematoxylin and eosin by Masson's trichrome method or by Mallory's phosphotungstic acid-hematoxylin technique.

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† With the technical assistance of Edward Iannucci, Peter Integlia, and Helen Cavallaro.

‡ Supported by grants from the Life Insurance Medical Research Fund, the Office of Naval Research, the American Heart Association, and the U.S. Public Health Service.

Received for publication August 7, 1957.

In addition to the morphological investigations, plasma lipids were estimated by standard techniques in several animals before the high-fat meal and thereafter immediately preceding the epinephrine injections. Chylomicrons were removed from plasma samples by high-speed centrifugation.

TABLE 1. THE EFFECT OF A HIGH-FAT MEAL ON THE PLASMA LIPIDS OF DOGS

Plasma sample	*Total cholesterol mg.%*	*Free cholesterol mg.%*	*Lipid phosphorus mg.%*	*Fatty acids mEq./l.*
Dog No. 2039 wt. 12.0 kg.				
1. Control, fasting	131.1	32.8	11.1	9.6
2. After fat meal	161.9	55.7	14.4	21.0
3. Sample #2, chylomicrons removed	148.7	39.3	16.0	15.0
Dog. No. 2040 wt. 7.5 kg.				
1. Control, fasting	151.1	36.9	11.2	10.0
2. After fat meal	181.5	39.9	13.8	19.0
3. Sample #2, chylomicrons removed	149.4	37.8	14.0	12.0
Dog No. 2086 wt. 10.0 kg.				
1. Control, fasting	94.6	23.1	11.2	13.3
2. After fat meal	124.5	31.5	13.0	24.3
3. Sample #2, chylomicrons removed	117.8	27.7	13.5	11.6
Dog. No. 2087 wt. 11.0 kg.				
1. Control, fasting	153.6	40.3	14.4	13.3
2. After fat meal	178.5	48.7	18.0	37.0
3. Sample #2, chylomicrons removed	157.7	39.9	16.4	15.0

RESULTS

The effect of a single high-fat meal on the plasma lipids of dogs.

Table 1 gives values for the plasma lipids of four dogs before and 3-5 hours after the feeding of the standard fat meal. The large increase in fatty acids is accompanied by lesser increases in values for cholesterol and phospholipids. Changes of this nature have been observed by others. (See Brun's review.[1]) Much of the cholesterol and fatty acid excess in the blood was associated with chylomicrons and could be removed with this fraction.

Changes in the coronary arteries and aortas of control dogs given high-fat meals alone or short hypertensive episodes alone.

The coronary arteries and aortas of five dogs fed single high-fat meals failed to reveal any lesions or lipid deposits.

As previously described, lesions of the coronary arteries and aortas occurred in animals subjected to a single, epinephrine-induced hypertensive episode. As early as 10-15 minutes after the epinephrine injections, hemorrhage into the medias of scattered small muscular coronary arteries was observed. After 1-3 days, edema and necrosis of medial muscle cells was present with or without accompanying medial hemorrhage (Figs. 1 and 2). The latter was sometimes massive. Of interest was the observation that the medial damage often began in or involved only the outer smooth muscle cells lying beneath the adventitia (Fig. 3). As the lesions progressed, this distribution in many led to perivascular inflammatory reactions rather than to subendothelial proliferation (Fig. 4). Associated thrombi were present occasionally.

Also of interest were the inflammatory exudates and the phenomena of repair associated with the lesions. In many injured segments the media was packed with red blood cells with little deposit of fibrin. In these lesions there was remarkably little cellular exudate. Indeed the paucity of inflammatory cells was a notable feature in many of the injured arteries. In other vessels smooth muscle necrosis was prominent rather than hemorrhage. Here the cellular exudate was also sparse and consisted of a few polymorphonuclear leucocytes and mononuclear cells (Fig. 7). Often the exudate was confined strictly within the boundaries of the media itself, but if the damage involved the outermost medial fibers, then a mild, radially arranged adventitial and periarteritic reaction developed. Occasionally the lesions exhibited during their acute stage in the media or adventitia accumulations of a "fibrinoid" substance. There was no suggestion that this material derived from primary alteration of the collagen of these regions. The impression was definitely gained that damage to smooth muscle was the primary event. Although the medial damage in these small arteries was great, no dilatation or aneuryism formation was noted.

Healing in the lesions took place by fibrosis within 1-3 weeks. Depending on the distribution and extent of the damage, this process was medial or medial and adventitial. Only rarely did the process result in a proliferative endarteritis. The few polymorphonuclear leucocytes were replaced by scattered mononuclear cells and the necrotic medial muscle and intravascular hemorrhage by scar.

The lesions in the coronary arteries that followed epinephrine-induced hypertension were distributed both in the right and left heart. The auricular vessels were also involved. In any one artery the damage was segmental and occurred frequently in relation to branches. While the lesions were most frequent in the intramyocardial divisions of the coronary arteries, many smaller epicardial branches were involved as well.

Fat stains were carried out on a large number of lesions from control dogs maintained on a low-fat laboratory diet and subjected to the usual hypertensive episode. Occasionally in the early stages of medial necrosis a very faint diffuse sudanophilia was recognizable. The positivity of this reaction in the control lesions was so slight as never to be confused with the intense sudanophilia of the lesions of the experimental animals.

Medial necrosis and hemorrhage in the aortas of dogs subjected to hypertensive episodes occurred frequently. These aortic lesions did not differ materially from those known for over 50 years to occur in rabbits' aortas following injections of extracts of the adrenal medulla.

Changes in the coronary arteries and aortas of dogs given a high-fat meal followed by a short, severe hypertensive episode.

The tissues of 24 animals were studied at intervals from a few minutes to three weeks after a large fat meal and arterial injury. The prior ingestion of a fat meal did not alter the distribution or incidence of the lesions of the coronary arteries. The outstanding difference between the arterial lesions of the fat-fed animals and those of the controls was the accumulation in the former of massive amounts of fatty substances. Lipids were not deposited in uninjured segments of arteries.

The accumulation of lipids within the injured arteries began promptly. Sudan IV preparations of the hearts of animals sacrificed within 30 minutes after the hypertensive episode revealed diffuse, intense sudanophilia of the dogs' plasma wherever it appeared within the lumens of veins or arteries. Where hemorrhage had occurred into the walls of the arteries, the injured segments were stained diffusely and intensely with the sudan dye, exactly like the plasma within the lumens of the vessels. The suggestion was inescapable that lipid-rich plasma had entered the vessel wall at the point of injury and was responsible for the diffuse sudanophilia in these zones.

Almost immediately the histological appearance of the lipid present in the injured arterial foci began to change. Even at one hour after injury brightly staining lipid droplets and granules larger than chylomicrons began to appear in the diffusely sudanophilic medial areas. These sudanophilic droplets increased in size and number progressively up to 24-48 hours (Fig. 5), during which time the dogs' plasma as viewed histologically

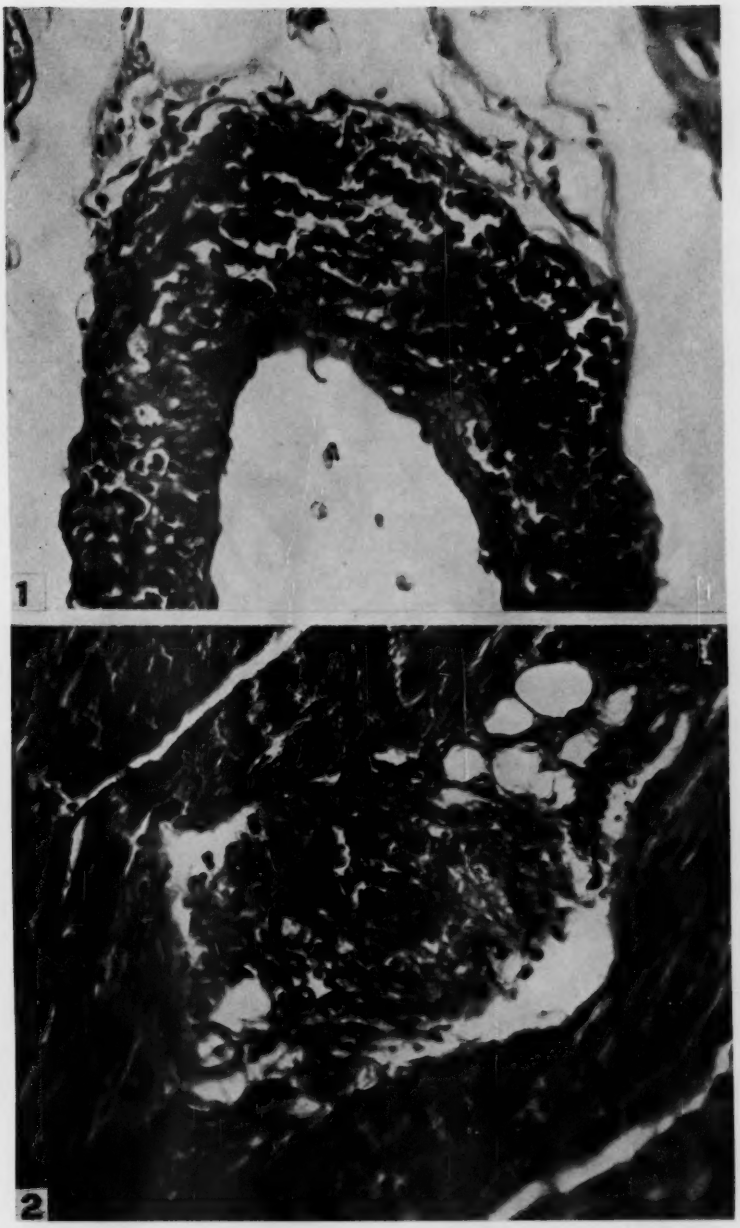

FIG. 1. Dog 1711. Cross-section of coronary artery four days after short, severe hypertensive episode. Massive hemorrhage in media. Little cellular exudate. x425.

FIG. 2. Dog 1712. Coronary arteriole 24 hours after hypertensive episode. Medial necrosis and perivascular cellular exudate. x325.

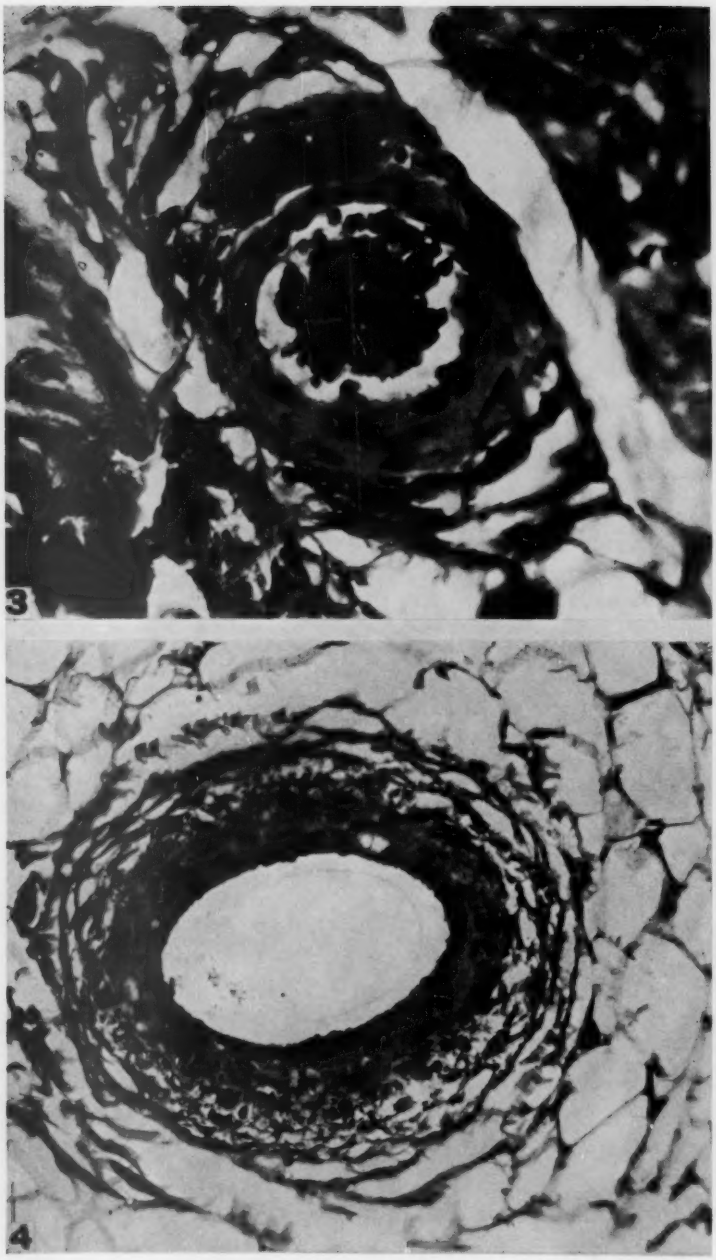

FIG. 3. Dog 1712. Coronary arteriole, 24 hours after hypertensive episode. Focal necrosis of outer media. x500.

FIG. 4. Dog 1716. Small epicardial coronary artery, six days after hypertensive episode. Necrosis of outer media with inflammatory reaction in adventitia. x325.

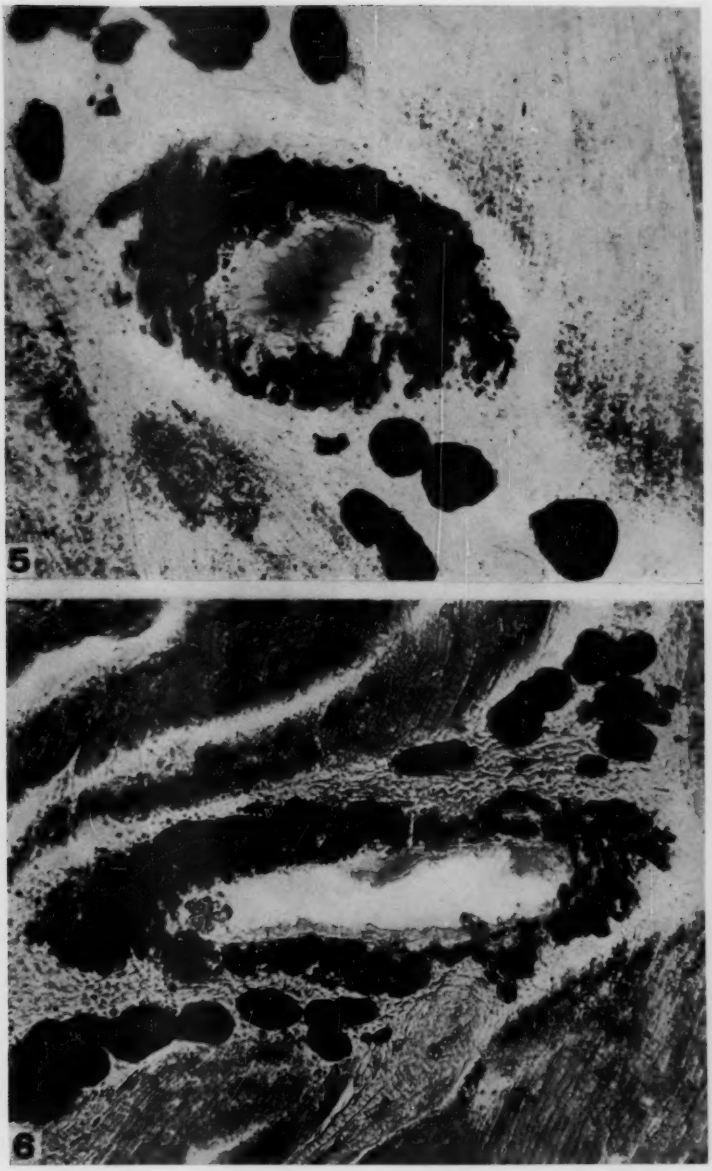

FIG. 5. Dog 2027. Coronary artery 24 hours after fat meal and hypertensive episode. Sudan IV stain, x350. Massive accumulation of lipid droplets in media. The plasma lipid stains diffusely in the lumen.

FIG. 6. Dog 1713. Coronary artery two days after fat meal and hypertensive episode. Sudan IV stain, x280. Massive deposit of lipid in media. Fat cells also are stained on either side of vessel.

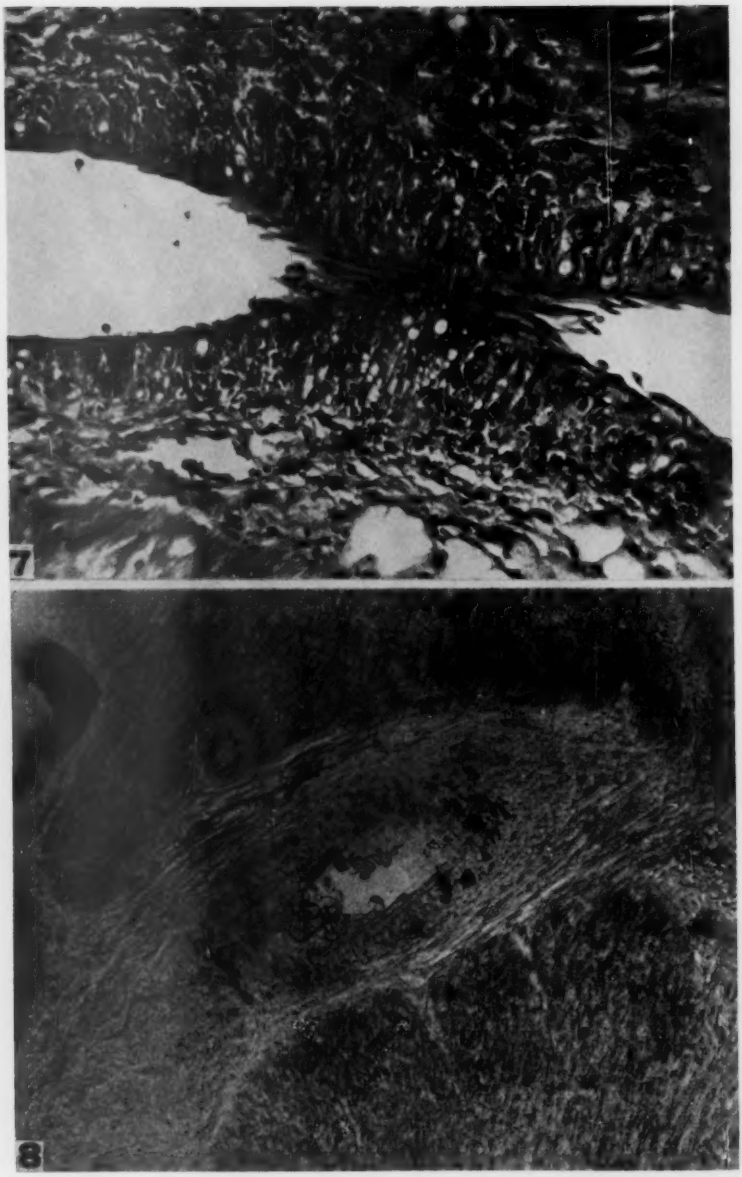

FIG. 7. Dog 1715. Tangential section of coronary artery at branch, six days after fat meal and hypertensive episode. Medial necrosis with sparse exudate. Note the numerous vacuoles representing extracellular lipid. x325.

FIG. 8. Dog 2099. Coronary artery 11 days after fat meal and hypertensive episode. Sudan IV stain, x250. Compare with Figures 5 and 6. The medial lipid has largely disappeared.

revealed a progressively decreasing diffuse sudanophilia. The lipoid droplets appeared to have accumulated and coalesced within the artery walls forming intensely staining granular masses that often obscured other medial structures (Fig. 6). The intramural lipid masses on occasion gave a faint positive Schultz reaction for steroids. In paraffin sections, routinely stained, there was little evidence of the deposited lipid in the early lesions. After several days, however, numbers of clear or partially clear medial vacuoles could be detected (Fig. 7). The presence of these relatively large amounts of lipid in the damaged media did not appreciably alter the morphological sequences already described in the lesions of control animals. After the 6th or 7th day, the lipid accumulations began to disappear (Fig. 8). The sudanophilic droplets and granular masses of lipid became progressively less and in animals sacrificed after three weeks had disappeared completely. Paraffin sections failed to reveal in the main the process by which this removal was accomplished. While an occasional monocyte with cytoplasmic vacuoles representing included lipid could be demonstrated, most of the fatty material appeared to remain extracellular in location. No foam-cellular or xanthomatous transformation of the lipid-containing medial foci occurred. The inflammatory exudates and the healing stages of the lesions were also not altered by the presence of lipids.

Lipid deposits similar to those in the coronary arteries and arterioles occurred in the injured segments of the aortas of dogs with alimentary hyperlipemia and epinephrine injections. Sequences of accumulation and disappearance of the deposited lipid followed the pattern described for the coronary arteries.

The effect of prior heparin administration on the accumulation of lipids at the sites of experimental arterial injury.

The promptness and massiveness of localization of lipids in the injured arterial walls and the absence of such deposits in lesions from control animals with nonlactescent plasmas suggested that the lipids being deposited derived from chylomicrons. It was thought of interest to determine whether or not heparin clearing of the lactescent plasma before arterial injury would prevent the accumulation of lipids in the artery wall. Consequently, six dogs were fed the standard high-fat meal. During alimentary hyperlipemia they were given 2 mgm./kg. heparin (Liquaemin sodium, Organon, Inc.) intravenously. Blood was obtained immediately after the injection and at five-minute intervals as necessary. When plasma clearing was complete, the hypertensive episode with epinephrine was instituted, followed by further blood sampling to make sure that chylomicrons did not reappear in the circulating blood. Usually they did not, and these animals

were sacrificed for histological examination from 1-72 hours after the hypertensive episode. In all of the animals hemorrhagic lesions of the small coronary arteries were present. The lipid component of the lesions was markedly altered. In no case was there accumulation of sudanophilic droplets at the sites of injury. At the earlier time intervals a diffuse sudanophilia of the lesions was present, coinciding with the increased sudanophilia of the plasma within the vessels' lumens. As the plasma staining became fainter, at longer time intervals, the sudanophilia of the lesions faded correspondingly and disappeared. No accumulations of lipid droplets or granular lipid masses were formed in the arteries.

The modification of intra-arterial lipid accumulation that followed the heparin clearing of plasma prior to injury was so striking that it prompted a test of the ability of the heparin system to clear lipid accumulations in arteries *after* injury.

The plasmas of a series of dogs were rendered lactescent by the standard high-fat meal. During the hyperlipemic state the animals' coronary arteries were injured in the usual way by injections of epinephrine. One hour following the hypertensive episode their plasmas were cleared by the intravenous injection of heparin. In some subjects of this series, complete clearing was not achieved, but in three animals the plasmas became limpid, and histological examination of the coronary arteries was carried out 1-3 hours after plasma clearing. No effect whatsoever could be detected on the lipids deposited in the injured arterial segments. In spite of the clearing of the ambient plasma, the lipid masses in the artery walls remained quite unaltered both in form and in amount.

It was of interest in contrast that clearing of lipids from the ellipsoidal arteries of the spleen occurred in the above animals. As is well known, after a high-fat meal, lipid accumulates in these fenestrated structures. Heparin clearing of the animals' lactescent plasmas either before or after epinephrine injury resulted in rapid clearing of the lipid deposits in the ellipsoids.

DISCUSSION

The foregoing experiments indicated that following a high-fat meal, massive lipid deposits occurred promptly and selectively at foci of acute injury in the coronary arteries and aortas of dogs. Intact vessels localized no lipid, emphasizing the importance of injury for this process. Evidence is presented that the accumulated lipid derived from the chylomicrons of the plasma. Thus localization of lipid did not occur in control animals with acute arterial injuries, but with nonlactescent plasmas. Further, it is known

that particulate substances, including other types of lipids,[2, 3, 4, 5, 9] accumulate, following their intravenous injection, at sites of acute arterial injury. Also, heparin clearing of the lactescent plasma before arterial injury prevented the accumulation of lipids in the vessel walls. It is unlikely that the lipid involved was plasma lipoprotein, as the content of molecularly dispersed lipid in the lactescent plasmas was essentially unchanged after the standard high-fat meal, at a time when lipid was accumulating in the arteries.

If it can be assumed that chylomicrons localized intra-arterially, it follows that these particles did not elicit phagocytic sequences in the arterial wall comparable to those occurring in arteriosclerosis in man. The lipid disappeared rapidly and apparently did not give rise to a foam-cellular xanthomatous response. This was in contrast to the fatty, foam-cellular arterial lesions that follow the combined injection of lipid-rich human plasma globulin and allylamine.[6] It must be pointed out, however, that the arterial lesions that follow allylamine injury differ from those associated with epinephrine administration in at least one important respect. The allylamine lesions usually include an extensive subendothelial proliferative component, whereas the epinephrine lesions exhibit little intimal proliferation and are mostly confined to the media. It would have been desirable to substitute allylamine injury for the epinephrine-induced hypertensive episodes in the present experiments, but this was not considered feasible because of the temporary anorexia produced in dogs by the administration of allylamine. Alimentary hyperlipemia would have been difficult to obtain during the several days needed for development of the allylamine lesions. The injured medias of dogs' small muscular coronary arteries or even the medias of dogs' aortas described in the present report cannot be considered to represent models of the intimal connective tissue of large elastic arteries in which arteriosclerosis in man develops. However, in acute arteriolar lesions in man, lipid is frequently found in the medias of the affected vessels. It is often in droplet form and is extracellular in location. Foam-cellular transformation of the media is rare. The experimental arterial lesions presented herein and the medial lipid-connective tissue reactions observed correspond more closely to certain stages of the medial lesions of human arteriolar necrosis and arteriolosclerosis than to the intimal, fatty disease of the larger vessels. Wilens has summarized well the possible relationships of these types of arterial lesions in man.[10]

Additional experiments revealed that the administration of heparin prior to the vascular-damaging hypertensive episode prevented the accumulation of lipids in the injured arterial segments. This emphasizes the importance

of the physical form of circulating lipids for their localization in the arterial wall. In the conditions of the experiment, the dispersed lipid-protein complex that is formed in heparin-cleared plasma did not localize in the arterial lesions. After the vascular injury, heparin administration, which often cleared the animals' plasma, did not effect the dispersal or removal of the aggregated lipid masses in the injured vessel wall. Apparently either the lipid particles no longer possessed the characteristics of chylomicrons, or plasma containing the clearing factor did not reach them. The ability of heparin to clear lipid accumulations from the ellipsoidal vessels of the spleen suggests that mere aggregation of chylomicrons in the vessel wall does not prevent their dispersal by the plasma-clearing system.

SUMMARY

Dogs were fed a large, high-fat meal. During the subsequent alimentary hyperlipemia, lesions of their coronary arteries and aortas were produced by a short, severe episode of hypertension induced by injections of epinephrine. Large quantities of lipids, probably derived from chylomicrons, accumulated selectively at the sites of arterial injury. This lipid did not elicit a foam-cellular phagocytic response that could be compared morphologically with arteriosclerosis in man. The lesions more closely simulated certain medial changes of arteriolonecrosis and arteriolosclerosis. The deposited lipid remained extracellular and for the most part in droplet form. It disappeared progressively from the vessel walls within two to three weeks. Clearing of the animals' lactescent plasmas with heparin before vascular injury prevented the accumulation of lipids at the subsequently injured arterial sites. Heparin administration after injury to arteries and the deposit of intramural lipids in them did not effect the removal of the fatty substances. Lipids accumulating in the ellipsoidal arteries of the spleen after a fatty meal disappeared on heparin clearing of the plasma.

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EXPERIMENTAL UNILOBAR PULMONARY EDEMA†

INTRODUCTION

In medical literature there are early accounts of what was probably acute pulmonary edema. In these early descriptions, however, it was confused with other catastrophic states manifested by acute cardio-respiratory symptoms. It was not until the eighteenth century that Albertini,[1] a student of Malpighi, made the first clinico-pathological correlations. He grouped chronic pulmonary congestion and pulmonary edema together but differentiated obvious right heart failure, hydrothorax, pneumothorax, and bronchial asthma. The belief that acute pulmonary edema was but a severe manifestation of congestive failure prevailed until the late 1930's when Farber,[5] Luisada,[9] Sir Thomas Lewis,[8] and others began to question this identity. Today, it is suspected by numerous workers that there are fundamental differences between acute pulmonary edema and chronic pulmonary congestion; however, the elements are obscure and often they blend into one another. The differences which exist may be considered under several headings:

First, as to *cause,* the pulmonary congestion seen as part of congestive heart failure is invariably due to heart disease, whereas acute pulmonary edema may result from numerous causes other than heart disease—toxic gases, drowning, uremia, central nervous system disorders, infections, thyroid storm, and allergy.

Second, in regard to the *clinical picture,* acute pulmonary edema is characterized by a very rapid onset and a severely cyanotic individual with copious, frothy, blood-tinged sputum who usually lacks the hepatic engorgement and peripheral edema which are seen in the patient with congestive heart failure, in whom also the onset is usually slower and the clinical picture not as severe. The latter usually makes a slow recovery only after treatment such as bed rest, digitalis, and mercurials, whereas the acute pul-

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† Project carried out under grants from the United States Public Health Service [H-1524 (c)] and the New Haven Heart Association.
Received for publication March 26, 1957.

monary edema patient may suddenly and dramatically improve or may die from asphyxia and shock.

Third, the *laboratory findings* disclose some differences, in that in acute pulmonary edema there is seen a lower blood oxygen saturation and the edema fluid is higher in protein than in congestive heart failure.

Because of the unpredictability of occurrence and severity of the patient's condition, few physiological observations have been made during actual attacks of pulmonary edema. Venous pressure is usually found to be high, and circulation time prolonged. Almost simultaneously with the onset of pulmonary edema there appears to be a generalized peripheral vasoconstriction associated with a great shift of circulating blood volume from the systemic circulation into the pulmonary circulation. Concomitantly, there is a rise in pulmonary vascular pressure. Some observers have felt that the peripheral vasoconstriction, by forcing excessive amounts of fluid into the lungs, is the *primary* event of acute pulmonary edema. As fluid begins to pour into the alveoli there is a corresponding rise in hematocrit, and some have claimed a drop in circulating blood volume, although the evidence for the latter is not clear. The cardiac output drops to very low levels and anoxia becomes marked, both the anoxic and stagnant types.

There are several areas in which the clinical and experimental data available today raise questions which remain to be answered.

1. What is the role of pulmonary vascular pressure? Two hundred years ago Stephen Hales[6] demonstrated that if water were perfused into the pulmonary artery, it would immediately appear in the bronchi, even under very low perfusion pressures. Very little would appear in the pulmonary veins. Conversely, citrated blood would appear in the veins but not the bronchi, even under high perfusion pressures. He concluded that the character of the blood and of the membranes determined the route of the fluid rather than the pressure.

2. What is the role of anoxia? Anoxia increases capillary permeability, and Drinker[4] attaches great importance to this. Certainly after edema has occurred, there is anoxia, but in the early stages its presence is not certain.

3. What is the role of the autonomic and central nervous systems? Under general or spinal anesthesia, pulmonary edema is uncommon, usually coming on, if it does occur, after recovery from anesthesia. Interruption of the cervical sympathetics or unilateral ablation of the corpora quadrigemina protects animals against pulmonary edema, and stellate ganglion block will occasionally protect human patients. Vagotomy, in some instances, will produce pulmonary edema.

4. What is the role of the pattern of ventilation? Again Stephen Hales observed that perfused fluid passed more easily from the pulmonary arteries

into the pulmonary veins when the alveoli were distended than when they were collapsed. Acute pulmonary edema is uncommon in obstructive emphysema where the lungs are overdistended.

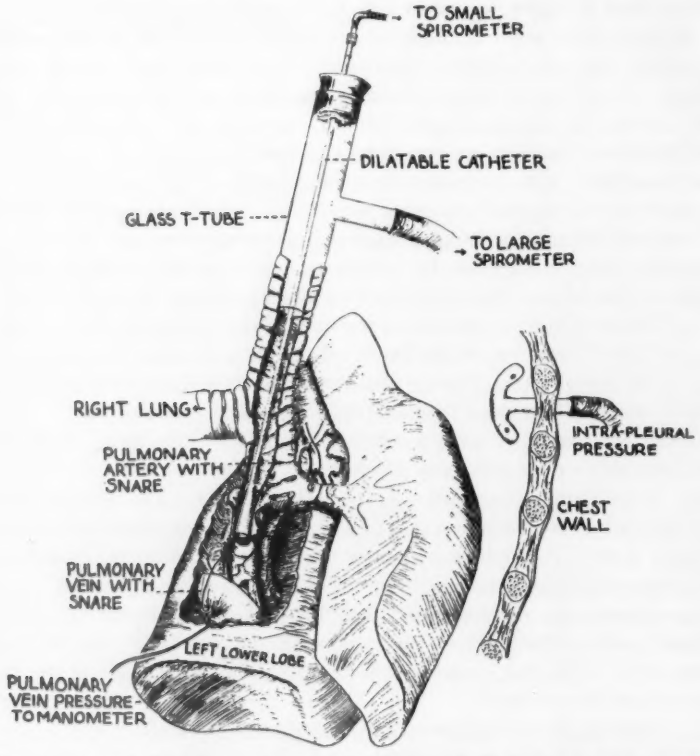

FIG. 1. Diagram of the experimental preparation. The left lower lobe pulmonary venous drainage is controlled by a snare loop, and a pressure recording catheter is in the pulmonary vein, peripheral to the point of occlusion. A similar arrangement exists, with snare around, and catheter (not shown) in the pulmonary artery. A ligature (not shown) secures the left lower lobe bronchus snugly around its cannula.

5. What is the relation between acute pulmonary edema and paroxysmal nocturnal dyspnea? The latter has some of the characteristics of both pulmonary edema and congestive failure. Is it a special manifestation of one or the other, or is it a combination of both?

6. What roles are played by some of the other factors beyond hydrostatic pressure, such as colloid osmotic pressure, lymphatic flow, and tissue

pressure? Rodbard[10] has suggested that in the case of the lung the latter is a function of intra-alveolar pressure, and possibly variations in this can occur by virtue of "atrial sphincters" producing trapping of air.

Extensive attempts have been made to study pulmonary edema by producing it experimentally in animals. Considerable species variation has been found. One of the methods which has been employed in the dog is the intravenous administration of normal saline solution. Volumes required have been such that, translated to a 70-kilogram human, 10 liters in five

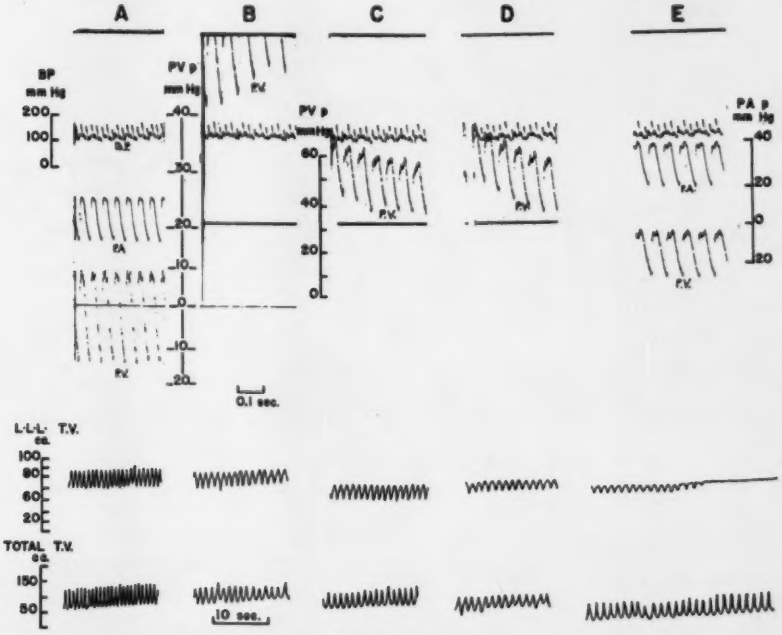

FIG. 2. Experiment 9. Showing the changes occurring in left lower lobe pulmonary vein pressure (PV_p) and left lower lobe tidal volume (LLL TV) under conditions of the experiment.

B.P.—femoral blood pressure.
PAp—left lower lobe pulmonary artery pressure.
Total T.V.—tidal volume of the remainder of the lung lobes.
Note the change in scale for PVp between B and C.

A. 12:45 p.m.—a baseline with lobar artery and vein occluded.
B. 12:52 p.m.—after infusion of 40 cc of blood into lobe via the pulmonary arterial cannula.
C. 12:54 p.m.—after 70 cc infusion. (Note change in PVp scale.)
D. 12:58 p.m.—after 130 cc infusion LLL TV reduced by one half in the presence of minimal change in total T.V.
E. 1:02 p.m.—after 170 cc infusion, showing final complete loss of LLL TV.

hours is the least quantity to produce pulmonary edema.[7] This degree of overhydration results in significant lowering of the hematocrit. Other methods of experimental production include severe degrees of intracranial trauma, as a result of which pulmonary edema may occur, often as the prelude to a fatal issue. To study the disorder as brought about by acute left heart failure, avulsion of aortic valve leaflets has been employed. Avulsion of one leaflet frequently causes pulmonary edema, but avulsion of two results in sudden death of the animal. Additional methods employed have

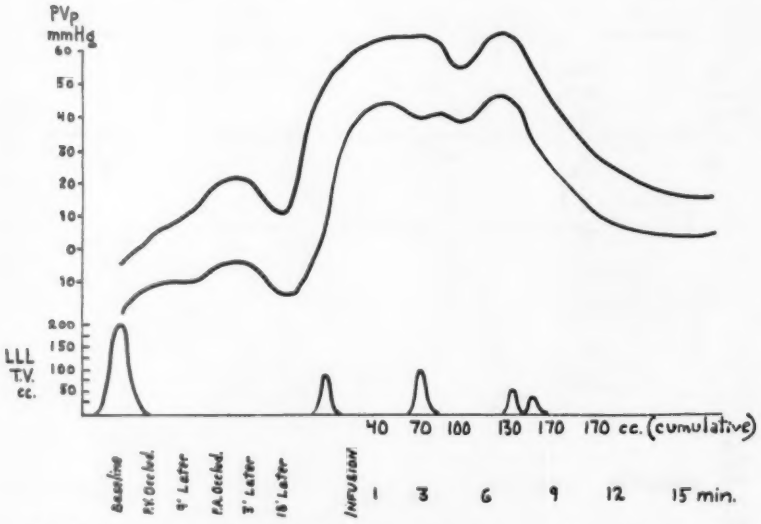

FIG. 3. Experiment 9. Graphic interpretation of experiment. Abbreviations same as in Figure 2. Under PVp, the upper line is the highest and the lowest line is the lowest value during a given pressure determination.

been the use of substances such as ANTU, ammonium ion, phosgene gas, or the injection of rather large doses of adrenalin.

It is apparent that most of the means available for the experimental production of pulmonary edema involve a severe degree of interference with vital processes, which means often that the short survival of the animal limits the number of studies possible. An objection to the saline overload pulmonary edema production is that it is only questionably comparable to the pathological state as it occurs in the human.

To eliminate some of these objections the present report describes an attempt to produce pulmonary edema in a single lung lobe of an otherwise

intact dog and to study the alterations in physiology associated therewith. It is believed that the lobe under observation represents pulmonary tissue with relatively intact innervation and bronchial arterial blood supply. It was hoped that studies on the unilobar level would permit a more clear-cut picture of the effect of alterations in vascular pressure and flow upon the aeration of lung tissue.

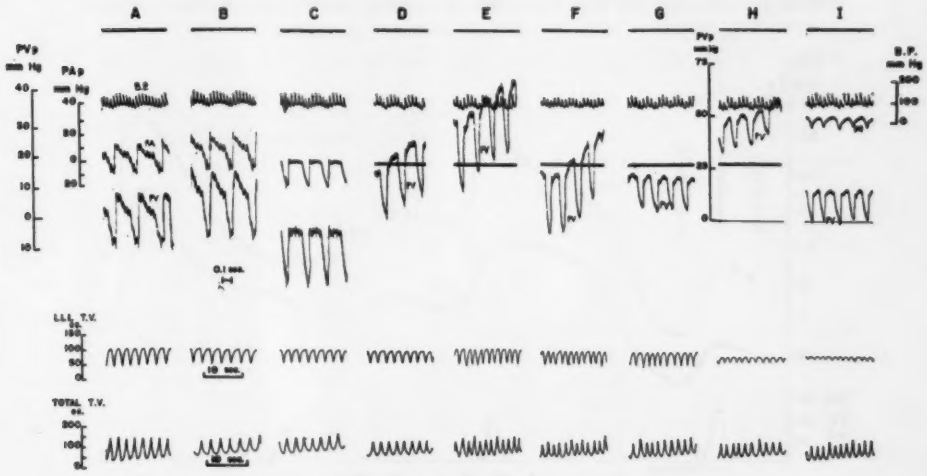

FIG. 4. Experiment 10. Abbreviations the same as in Figure 2.

A. Baseline
B. 8 minutes after PV occlusion.
C. 12:35 p.m. 8 minutes after PA occlusion.
D. 12:46 p.m. After infusion of 20 cc blood.
E. 12:40 p.m. After 120 cc.
F. 12:52 p.m. After 240 cc.
G. 12:55 p.m. After 360 cc.
H. 12:59 p.m. After 450 cc. LLL TV markedly decreased.
I. 1:03 p.m. After 460 cc. Further decrease.

METHOD

The experiments were acute in nature and were carried out on large dogs anesthetized with Nembutal. Tracheotomy was performed as the first step and a positive pressure apparatus was connected to maintain respiration during operation. Via a left thoracotomy the artery, veins, and bronchus of the left lower lobe were identified. Snare loop cannulae were prepared which would permit occlusion and release of vessels from outside after closure of the chest.[11] One of these was placed around the right lower lobe artery and one around the vein or veins (Fig. 1). Fine polyethylene cannulae for pressure recording were led into the lobe, one via the artery and one via the vein, in such a manner that they were not compressed by occlusion of the snare

loops. A cannula was passed down the trachea into the left lower lobe bronchus where it was secured in an air tight manner by an encircling ligature. A metal cannula was passed through an interspace for pleural pressure measurement and then the thoracotomy wound carefully closed in layers. The positive pressure apparatus was disconnected and recordings begun. The left lower lobe cannula was connected to a small Krogh spirometer, and a spirogram of the remainder of the lobes obtained by a bell spirometer. The lobar artery and vein catheters were connected to a Hathaway multichannel inductance-type pressure recorder for measurement of intravascular pressures. The same apparatus was used to record intrapleural pressure and femoral arterial

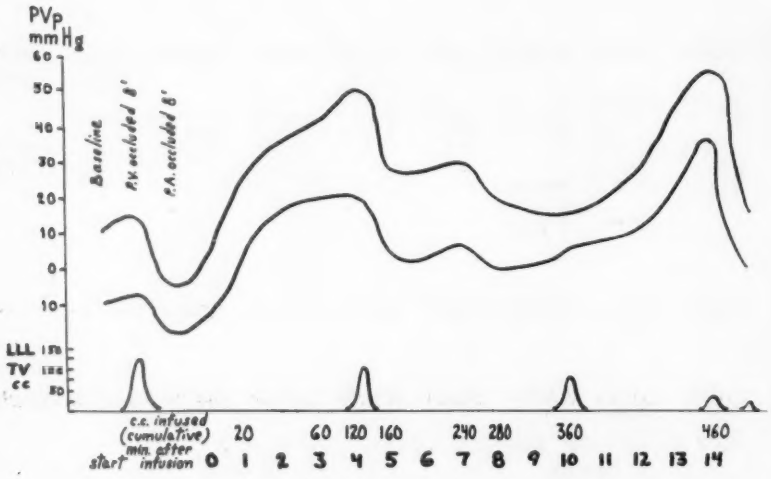

FIG. 5. Experiment 10. Graphic interpretation of experiment. Legends as in Figure 2.

pressure. Baseline readings were taken and then the lobar artery and vein snare loops separately tightened, occluding the respective vessels. After the animal had adjusted to these manipulations, femoral arterial blood was collected in a Fenwal (R) plastic bag containing acid-citrate-dextrose as anticoagulant. This manner of collection avoids contact of the blood with room air. With the lobar artery and veins occluded, this blood was then infused into the left lower lobe via the pulmonary arterial catheter, the tip of which lay distal to the point of occlusion by the snare cannula. This meant that the only egress of blood from the lobe that could occur was a small amount via bronchial veins. The tip of the lobar pulmonary vein catheter lay distal to the point of occlusion by the venous snare, thus permitting measurement of the pressure levels reached during infusion into the sequestrated lobe. Infusion was continued until changes in the left lower lobe spirogram suggested a significant degree of diminution of the tidal exchange. Attention was directed to the correlation of these changes in the lobar tidal volume with the pulmonary vein pressure.

The experiments were terminated by exsanguination and post-mortem examination was carried out to ascertain correct placement of the cannulae and snare loops.

Matched portions of right and left lower lobes were submitted for histological examination. It is believed that in a lobe prepared in this manner, the neural and bronchial arterial supply remains largely intact.

RESULTS

No consistent changes in the ventilatory pattern of the left lower lobe occurred from simple occlusion of lobar artery or vein in varying combinations. Infusion of fresh blood into the artery, however, with the vein occluded almost uniformly resulted in changes in the respiratory pattern, namely, a gradual diminution in the tidal exchange, progressing to complete

TABLE 1. PULMONARY VENOUS PRESSURE (PV_p) LEVELS AT WHICH PULMONARY EDEMA OCCURRED

Experiment No.	PV_p,* *mmHg*
8	24/18
14	12/12
20	20/0
22	26/?
26	24/4
12	40/20
13	40/18
17	40/32
18	40/12
9	65/45
10	60/40

* Pressures expressed as highest valve in each tracing over lowest.

suppression of this exchange if large amounts of blood were infused (Figs. 2-5). Often during the infusion an acceleration of the animal's respiratory rate would occur, the implication being that neural connections to the lobe were intact, permitting a reflex tachypnea to occur (Churchill-Cope Reflex).[8]

Interest centered upon the levels to which pulmonary vein pressure would rise under conditions of this experiment since little is known of the exact relationships between intrapulmonary vascular pressures and the development of pulmonary edema. To accomplish this an arbitrary degree of diminution of lower lobe tidal volume was selected as that point at which pulmonary venous pressures would be compared. It was believed that a reduction of tidal volume by *one half of baseline* value would be comparable with a significant degree of clinical pulmonary edema. A comparison was

made among the various experiments of the intralobar venous pressure reached at that point when lobar tidal volume had been reduced to one-half of its baseline value. Table 1 shows these comparisons. The vascular pressures are expressed in terms of millimeters of mercury, with the highest value during a given pressure tracing over the lowest.

To be certain that the changes in the left lower lobe spirogram were not merely secondary to any over-all changes in the animal's respirations, the respiratory pattern of the remainder of the lobes was observed as measured by the bell spirometer. Intrapleural pressure recordings during the course of the experiment did not reveal changes of significance, although in some instances respirations became more labored during the induced vascular congestion of the left lower lobe, and this was reflected in greater negative pressure during inspiration.

The sequence of events was of interest in that as increments of blood were infused into the lobe, rather high pressures were reached before any pulmonary edema became manifest, but then, with the beginning of marked diminution of lobar tidal exchange, the lobar venous pressure would drop.

The histological sections showed consistently interstitial and intra-alveolar edema and hemorrhage, varying with the amount of blood infused. In some experiments infusion into the lobe was continued to a point where respiratory exchange ceased completely, and in these the histological picture was more extreme.

DISCUSSION

It is believed that this series of experiments represents a small fraction of what may be learned from the study of pulmonary edema on the unilobar level. The results are of interest in two regards. First of these is the figure for the *height* of pulmonary vascular pressure reached during the experiments before significant pulmonary edema occurred, as herein defined. Figures of 40 to 60 mm of mercury, as found in some of these experiments, are somewhat higher than others have stated for the development of pulmonary edema.

Second, it is of interest to note that there is *no* constancy of the lobar venous pressure levels at which edema occurs. In some experiments it appeared in the 20 mm Hg range, whereas in others, levels of 40 or 60 were required (Table 1). This finding suggests corroboration of the viewpoint of Altschule[8] and others that pulmonary edema is not purely a function of pulmonary venous pressure, but that other factors are operative, such as lymphatic flow, and possibly changes in intra-alveolar pressure. Rodbard has discussed the possible significance of the latter.[10]

An additional possibility which presents itself is that the rather large volumes of blood which the lobe can accommodate before edema occurs is a function of expansion of the infused lobe at the expense of the others. No confirmation of this possibility is available at present.

These experiments point the way toward more detailed studies which are currently in progress concerning the exact manner in which vascular congestion alters the respiratory dynamics of the lobe. What is the relative degree of impairment of inspiration versus expiration? What changes in pulmonary compliance occur under conditions of this experiment? These are questions which remain to be answered.

SUMMARY

1. A series of experiments is described wherein pulmonary congestion and/or edema are studied in a single lobe of a relatively intact dog.

2. This lobar hypervolemia is produced by infusion of blood into a lobe, the arterial inflow and venous outflow of which are occluded.

3. Rather high levels of pulmonary venous pressure may be reached before pulmonary edema occurs.

4. There is not a constant relationship between pulmonary venous pressure and the development of edema under the conditions of the experiment.

5. It is suggested that the findings lend support to the concept that pulmonary edema should not be considered entirely in terms of left ventricular failure and changes therefrom in pulmonary venous pressure.

6. The direction of further studies is mentioned.

ACKNOWLEDGMENTS

Gratitude is expressed to Dr. Gustaf E. Lindskog for providing the stimulus which led to this work. Gratitude is expressed also to Dr. Averill A. Liebow for helpful suggestions. The technical assistance of Dr. Charles L. Rickerich and of Mr. Armand Negri is acknowledged.

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The Board of Editors

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THE EFFECT OF ESTROGEN ON CARTILAGE AND BONE IN CASTRATE C_3H MICE†

The contrasting effects of estrogen upon the pubic symphysis and long bones in mice is well known.[10, 17, 18, 25] At the time new bone was forming in the long bones the number of reticular fibers increased in the marrow.[10] Because observations were made only after 30 days following implantation of estrone, it was not possible to determine whether these fibers were associated with the formation of new trabeculae of bone or were remnants of trabeculae that were undergoing resorption and reconstitution. Glycogen, alkaline phosphatase, and calcium salts were demonstrated in the reticular stroma of newly forming membranous bone in pig embryos.[3] Therefore, it seemed profitable to study the distribution of these substances as well as reticular fibers in the long bones after short periods of estrogen treatment in order to determine whether or not an analogous process occurs in the development of endosteal bone in response to estrogen.

Considerable data are available on the chemical composition and histochemical properties of the guinea pig symphysis during pregnancy and after treatment with estrogen and relaxin.[4, 14, 15, 20, 29, 30, 33, 37, 38] The mouse symphysis has been studied histochemically only with toluidine blue and PAS during pregnancy,[28] and with toluidine blue alone in castrate mice receiving hormonal treatment.[28] In the present experiment, the knees and the pubic symphyses of castrate mice were examined with the use of histochemical methods at daily intervals during a 30-day period of estrogen treatment in order to trace and compare the changes in response to estrogen at these two sites.

MATERIALS AND METHODS

One hundred twenty-two male and female mice of the C_3H strain were castrated at 3 weeks of age. The experiment was delayed until the mice were 6 to 9 weeks of age.

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† The material here presented is taken from a thesis submitted to the Faculty of the School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine. The research was performed during tenure of a Fanny Bullock Workman Scholarship and Horton-Hallowell Fellowship of Wellesley College, and of a James Hudson Brown Memorial Summer Fellowship of Yale University School of Medicine.

Received for publication July 3, 1957.

Half of the mice of each sex received no treatment. The remaining mice received 1.5 μg of estradiol benzoate dissolved in 0.03 cc. of sesame oil* daily. One untreated and one estrogen-treated mouse of each sex was sacrificed each day for 30 days. Necropsy was performed 24 hours after the last estrogen injection. All mice were given Purina Fox Chow and water ad libitum throughout the experiment.

The dissection and examination of the pubic symphyses and knees at necropsy were carried out as previously described.[5, 10] Flexibility of the symphysis was graded from 0 to 5+. The tissues were fixed in chilled 80 per cent alcohol for 24 hours, then dehydrated rapidly and embedded in paraffin at 60° C. under partial vacuum. The tissues were stored at 4° C. until they were sectioned in order to prevent the deterioration of the alkaline phosphatase.

By coating the face of the block with melted paraffin before cutting each section, undecalcified knees and symphyses were sectioned at 14 mμ. Sections of the knee and the symphysis were mounted together on glass slides using Mayer's albumin without thymol as an adhesive agent.

The tissues from each animal were stained by five different methods:

1. *The Von Kossa method for demonstration of mineral salts.*[27]

2. *Laidlaw's silver method for the demonstration of reticular fibers.*[9] The ferric ammonium sulfate sensitization recommended by Gomori[10] was added to the standard procedure.

3. *The Gomori method for demonstration of alkaline phosphatase.*[8] Three slides from each animal were stained by this method. All sections were decalcified for 40 minutes in formic acid-sodium citrate buffered at pH 5.0 prior to staining for alkaline phosphatase in order to eliminate confusion between sites of preformed calcium salts and sites of alkaline phosphatase activity. One slide from each animal was used as a control and was incubated for 120 minutes in buffer without substrate. A second slide from each animal was incubated in buffered substrate for 10 minutes, while a third was incubated in buffered substrate for 120 minutes. All slides were collodionized after decalcification in order to facilitate preservation of the sections during incubation.

4. *The toluidine blue method for demonstration of metachromasia of the cartilage matrix.*[28] One slide from each animal was collodionized and stained without preliminary treatment. A second was decalcified for 40 minutes in formic acid-sodium citrate buffered at pH 5.0 prior to staining.

5. *The Hotchkiss modification of the periodic acid-Schiff method for demonstration of polysaccharides.*[1, 2, 24] Three slides from each animal were stained by this method. One was digested for one hour at 37° C. in buffered 1 per cent diastase to remove glycogen prior to staining. A second was stained directly without preliminary digestion. The third slide in half of the animals was digested for one hour in buffer without diastase prior to staining. The third slide from the remaining animals was used as a control by omitting the oxidation in periodic acid prior to staining.

After staining, the slides were rearranged so that all of the slides from each animal were together. During the initial reading the slides were identified only by sex and number, so that the treatment which an animal had received was unknown to the observer at the time the observations were made.

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* Injected sesame oil has no effect on the skeleton in mice.[35]

OBSERVATIONS

THE PUBIC SYMPHYSIS IN UNTREATED MICE

The pubic symphyses in untreated mice of both sexes were only slightly flexible. The interpubic separation was 0.25 mm. or less. The uterus was atrophic with two exceptions in which it was slightly enlarged but not as distended as in an estrogen-treated animal. The seminal vesicles were atrophic. The bladders were not distended or hypertrophied in the untreated animals.

Microscopically, the central portion of the pubic symphysis of the untreated mice consisted of fibrocartilage which was continuous with the articular caps of hyaline cartilage covering the medial surfaces of the pubes. Most of the symphyses contained a midline cleft in the cranial portion of the symphysial fibrocartilage (Fig. 1). Adjacent to the cleft, the chondrocytes were close together in flattened lacunae with their long axes directed along the sagittal plane. Each lacuna contained one or at the most two chondrocytes. In the hyaline cartilage the chondrocytes were grouped into nests of 6 to 8 cells in semi-columnar arrangement. The cartilage of the pubes adjacent to the marrow consisted of hypertrophic and calcified cartilage supported by slender trabeculae of cancellous bone. The trabeculae and surface of the calcified cartilage were lined with osteoblasts. Dilated sinusoids were frequently present at the junction of the marrow and the calcified cartilage matrix. The marrow was hemopoietic.

Coronal sections through the cortical portion of the pubic bone adjacent to the symphysis showed occasional small indented areas in the bone where the symphysial cartilage blended with the periosteum of the pubes at the cranial and caudal ends of the symphysis. These areas contained a network of reticular and collagen fibers which blended with those of the periosteum. Collagen or reticular fibers extended between the cartilage and pubic bones only in these small areas.

The trabeculae and cortical bone of the pubes and the calcified cartilage adjacent to the marrow contained calcium salts. Calcium salts were also demonstrated in islands of hypertrophic and calcified cartilage which were frequently present in the medial-most portions of the hyaline cartilage of the pubes. There were no other deposits in the fibrocartilage, hyaline cartilage, or connective tissue of the symphysis.

Alkaline phosphatase was demonstrable in sections incubated for 10 minutes in the osteoblasts surrounding the cancellous bone and the calcified cartilage, in the chondrocytes in the zone of hypertrophic and calcified cartilage next to the marrow, and in the chondrocytes of the islands of hypertrophic and calcified cartilage in the medial portions of the articular caps.

Neither the chondrocytes nor the matrix of the hyaline cartilage showed activity.

The central fibrocartilage was metachromatic in 15 of the 20 males and 10 of the 13 females from which sections through this area were stained with toluidine blue. The cranial portion of the fibrocartilage near the central cleft was most metachromatic. Caudally, where the fibrocartilage merged with the connective tissue of the caudal part of the articular capsule, the tissue was orthochromatic (Fig. 1). The hyaline cartilage was strongly metachromatic. Calcified cartilage was not metachromatic in undecalcified sections but was intensely metachromatic after decalcification. The bone and connective tissue stained orthochromatically in both calcified and decalcified sections.

The matrix of the central fibrocartilage and of the nonhypertrophic and hypertrophic hyaline cartilage of the articular caps stained light pink with PAS. The staining of the matrix did not decrease after digestion with diastase. Bone matrix and collagen stained faint pink. Glycogen was inconstantly present in the chondrocytes of the central fibrocartilage: large amounts were present in the sections from 10 of 21 males and 13 of 24 females that passed directly through the central fibrocartilage. Those sections in which glycogen was not detected in the chondrocytes of the central fibrocartilage also showed smaller amounts of glycogen in the chondrocytes of the hypertrophic cartilage. Glycogen was usually present in the chondrocytes of the fibrocartilage adjacent to the hyaline cartilage caps of the pubes, and its matrix stained similarly to that of the central fibrocartilage except for a gradual decrease in intensity toward the caudal end of the symphysis. The chondrocytes of the hyaline cartilage caps contained less glycogen and the matrix stained slightly less intensely than that of the fibrocartilage and the hypertrophic cartilage. The chondrocytes of the hypertrophic cartilage contained large amounts of glycogen, but those of the calcified cartilage contained little or no glycogen. Chondrocytes in the islands of calcified cartilage in the caps of the pubes showed a similar pattern: chondrocytes in the early stages of hypertrophy contained more glycogen than those in the surrounding fibrocartilage, while those immediately adjacent to the calcified matrix contained the most glycogen. The cytoplasm of chondrocytes surrounded by calcified matrix was vacuolated and contained little or no glycogen.

THE PUBIC SYMPHYSIS IN ESTROGEN-TREATED MICE

As the tissue changes progressed in the symphysis during estrogen treatment, the joint was technically no longer a symphysis but will be referred to as such to avoid ambiguity.

The symphyses of the estrogen-treated mice during the first several days of treatment were slightly more flexible than those of the untreated mice. On the 6th day, the female showed a 4+ symphysial flexibility and an interpubic distance of 0.5 mm., with slight resorption along the medial margins of the pubic bones. The males showed slight increased symphysial flexibility and inconstant resorption of the medial margins of the pubes between the

TABLE 1. THE RESPONSE TO ESTROGEN AT THE PUBIC SYMPHYSIS AND THE KNEE JOINT

Days of treatment	*Flexibility*		*Interpubic gap, mm.*		*Knee trabeculae*		*Reticular fibers*	
	males	*females*	*males*	*females*	*males*	*females*	*males*	*females*
1	1+	1+	<0.25	<0.25	N	N	A	A
2	1+	1+	<0.25	<0.25	N	N	A	A
3	1+	1+	<0.25	<0.25	N	N	A	A
4	1+	1+	0.25	0.25	N	N	T	A
5	1+	2+	0.25	0.25	N	N	A	T
6	2+	4+	0.25	0.50	1+	1+	P	P
7	1+	4+	0.25	0.25	1+	1+	P	P
8	2+	4+	0.25	0.50	1+	1+	P	P
9	2+	4+	0.25	0.50	1+	1+	P	P
10	3+	4+	0.25	0.75	1+	1+	A	P
11	4+	4+	0.75	0.75	2+	1+	P	P
12	3+	4+	0.50	0.75	2+	2+	P	P
13	4+	4+	0.50	0.75	3+	4+	P	P
14	3+	4+	0.25	0.75	1+	3+	P	P
15	4+	3+	0.50	0.25	2+	3+	P	P
16	4+	4+	0.50	0.75	4+	4+	P	P
17	3+	4+	0.25	0.75	2+	4+	P	P
18	4+	5+	0.25	1.00	4+	4+	P	P
19	4+	5+	0.50	1.00	4+	3+	P	P
20	3+	5+	0.50	1.00	4+	4+	A	P
21	5+	5+	0.75	1.00	3+	4+	P	P
22	4+	5+	0.50	1.00	4+	4+	P	P
23	5+	5+	0.50	1.00	4+	4+	P	P
24	4+	5+	0.50	1.00	4+	4+	P	P
25	5+	5+	0.75	1.25	4+	4+	P	P
26	4+	5+	0.75	1.50	4+	4+	P	P
27	4+	5+	0.75	1.25	4+	4+	P	P
28	5+	4+	1.00	0.75	4+	3+	A	P
28	4+	4+	0.50	0.50	4+	4+	P	A
29	4+	5+	0.50	1.00	4+	4+	P	A
30	4+	5+	0.75	1.00	4+	4+	A	P

A—Absent. N—Normal. P—Present. T—Trace.

6th and 11th days, but did not show a response comparable to that in the females until the 11th day (Table 1). The greatest amount of flexibility in the females occurred by the 18th day and in the males by the 19th day. Males treated from 19 to 30 days had an average flexibility of 4+ and an interpubic ligament averaging 0.6 mm. in length. Females treated for the same length of time showed a flexibility of 5+ and an interpubic ligament averaging 1.0 mm. in length. The uteri were increased in size from the 1st to the 30th day of estrogen treatment. The seminal vesicles showed moderate enlargement after 4 days of treatment and marked enlargement after 10 days. None of the females had distended bladders. The response of the bladders of the males was inconstant but distension was seen most frequently after 17 days of treatment.

The histological response to estrogen was similar in both sexes except that the males responded more slowly and to a lesser degree than the females. The morphological changes occurring in the two sexes will therefore be described together. The pubic symphyses of the estrogen-treated mice resembled those of the untreated mice during the first 2 to 3 days of treatment. After 3 to 4 days the matrix of the hyaline cartilage caps of the pubes was swollen and the chondrocytes were in random rather than semicolumnar arrangement. On the 5th day, rounding of the chondrocytes and increased cellularity of the central fibrocartilage were present. Many lacunae contained 2 to 4 rather than 1 to 2 chondrocytes. By the 6th day, there was resorption of the bone at the cranio- and caudo- dorsal ends of the symphysis at the sites where there was normally a slight indentation of the margin of the bone in the untreated mice. The bone and marrow normally adjacent to the calcified cartilage matrix were partially replaced by a mass of highly cellular connective tissue which extended inward toward the center of the symphysis from its cranial and caudal ends. The cells resembled fibroblasts. The surface of the bones adjacent to the connective tissue was irregularly eroded and osteoclasts were present in eroded lacunae of the bone and along the surface of the calcified cartilage next to the connective tissue. The eroded areas contained many fine reticular fibers, in contrast to the heavier collagen fibers of the normal periosteum. Extension of the connective tissue inward between the cancellous bone and the symphysial cartilage progressed in extent throughout the period of treatment. By the 19th day it was interposed between the bone and the cartilage for the entire length of the symphysis.

On the 8th day the central fibrocartilage resembled hyaline more than fibrocartilage. A few flattened chondrocytes still persisted along the midline in the male. The process of "hyalinizing" of the fibrocartilage matrix and

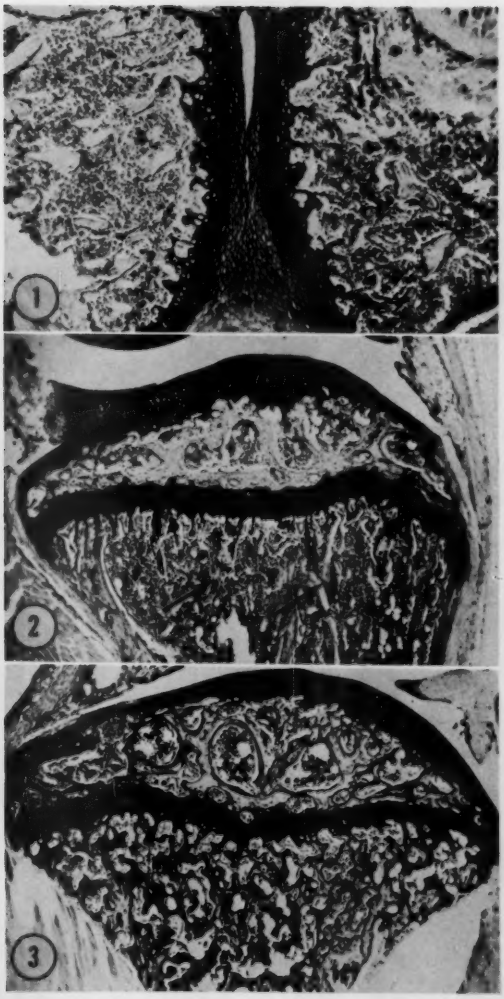

FIG. 1. Decalcified coronal section of the symphysis pubis from an untreated castrate male stained with toluidine blue. The metachromasia, which is most pronounced in the fibrocartilage surrounding the central cleft, gradually diminishes caudally. x32.

FIG. 2. Decalcified sagittal section of the proximal end of the tibia from an untreated castrate male. Trabeculae (arrows) in the metaphysis are slender and oriented parallel to the long axis of the bone. Toluidine blue stain. x27.

FIG. 3. Decalcified sagittal section of the proximal end of the tibia from a castrate female treated with estrogen for 30 days. The numerous trabeculae in the metaphysis are oriented at random rather than parallel to the long axis of the bone. Toluidine blue stain. x27.

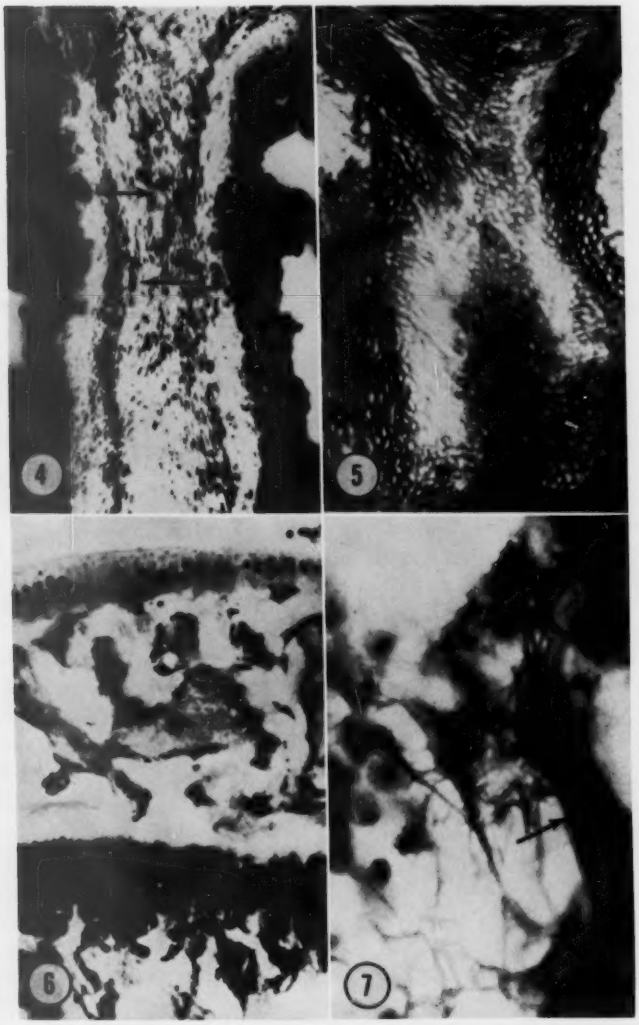

FIG. 4. Decalcified coronal section of the symphysis pubis from a castrate female treated with estrogen for three days. Alkaline phosphatase is present in many of the chondrocytes of the central fibrocartilage (arrows). x70.

FIG. 5. Undecalcified coronal section of the symphysis pubis from a castrate female treated with estrogen for 22 days. Part of the cartilage caps of the pubes remain. The central portion of the symphysis is composed of a tissue intermediate between cartilage and connective tissue. Laidlaw's silver stain. x75.

FIG. 6. Decalcified sagittal section from the proximal end of the tibia of an untreated castrate female. Gomori method for alkaline phosphatase. x79.

FIG. 7. Undecalcified section of the tibial marrow from a castrate male treated with estrogen for four days. Reticular fibers form a network projecting into the marrow from the surface of the trabecula (arrow). Laidlaw's silver stain. x1250.

rounding of the lacunae with multiplication of the chondrocytes continued progressively throughout the period of treatment. By the 10th day the cartilage near the midline consisted of numerous cells with full oval to round nuclei set in a glassy homogeneous ground substance in which no fibrillar structure was visible in sections stained with toluidine blue or PAS. Cellularity was greatest at the center of the symphysis; toward the periphery the chondrocytes were separated by wider intervals of homogeneous matrix. After 14 days a few areas were present in which the silver stain showed remnants of cartilage matrix and lacunae superimposed upon thick bundles of collagen. After 16 to 17 days, silver stains showed many areas of the central symphysial cartilage to be intermediate between cartilage and connective tissue (Fig. 5). In transitional areas, the collagen bundles nearest to the cartilage formed lacuna-like spaces around the cells. This orientation gradually changed to mere undulations of the collagen fibers and finally to straight bundles of collagen with no lacunae around the fibroblasts in areas of connective tissue. The cells were identical in appearance in areas where no matrix could be demonstrated either with the silver stain or with PAS. Occasional areas of cartilage in which the lacunae were elongated as in fibrocartilage of untreated mice persisted as late as the 23d day, particularly in the more cranial portion of the symphysis. The matrix in these areas was, however, hyalinized. As the length of treatment progressed, the remnants of cartilage decreased in amount until after 25 to 30 days of treatment the interpubic tissue consisted of a large area of connective tissue in which the remains of the hyalinized cartilage were located either centrally or to one side.

There was no change in the distribution of calcium salts in the symphyses of the estrogen-treated mice as compared to the untreated mice. Isolated remnants of unabsorbed calcified cartilage occasionally remained in animals treated for 20 days or more.

Alkaline phosphatase was present in all of the sites in which it was present in the untreated animals, and in addition, was present in the central fibrocartilage and connective tissue of the caudal portion of the articular capsule after the 3d day of treatment (Fig. 4). Activity was most prominent in areas which were most highly cellular: in the midline region of the symphysis, and at the junction of this midline region with the caudal connective tissue of the articular capsule. There was heavy activity in the areas where the connective tissue extended inward from the periosteum between the cancellous bone and the calcified cartilage. Activity was maximum along the line of the eroded bone surface and along the surface of the calcified cartilage matrix in regions which were richest in fine reticular fibers and highly

cellular connective tissue. This general pattern, established by the 6th to 7th day, continued throughout the period of treatment.

Metachromasia of the central fibrocartilage was not present after the 9th day in females and after the 14th day in males. Fading began at the caudal end of the symphysis and gradually worked cranially so that the extreme cranial portion of the symphysis was the last to lose its metachromasia. In animals treated longer than 14 days, metachromasia appeared only in remnants of hyaline cartilage and in remnants of calcified cartilage matrix in decalcified sections.

The intensity of staining of the symphysis with PAS was not decreased after estrogen treatment. Glycogen was present in the chondrocytes of the central cartilage in 6 of 7 males in which appropriate sections were stained during the first 9 days of treatment, and in all of the females during the first 4 days of treatment. After the 9th day, there was no glycogen in the chondrocytes of either the central or the hyaline cartilage in any of the 15 males in which sections passed through areas in which glyocgen was normally present. After the 5th day, no glycogen was present in any of 17 females in which the sections passed through normally glycogen-containing areas. Some glycogen was present in a few of the chondrocytes in the hypertrophic cartilage after 18 days of treatments, although the amount was generally less than was found in the untreated animals.

THE KNEE IN UNTREATED MICE

The articular surfaces of the femur and tibia consisted of hyaline cartilage supported by a thin layer of bone. Between the hyaline cartilage and the bone there was a narrow layer of calcified cartilage. The epiphysial plates consisted of a narrow layer of hyaline cartilage supported on its epiphysial surface by a thin layer of bone, a layer of columnar cartilage averaging 8 cells in thickness, and a layer of sclerotic cartilage 2 to 4 cells in thickness. The metaphysis contained hemopoietic marrow and slender trabeculae of bone oriented parallel to the long axis of the bone (Fig. 2). There were no reticular fibers projecting from the surfaces of the bony trabeculae or from the endosteal surface of the cortical bone.

Mineral salts were demonstrable in the trabeculae of the epiphysis and metaphysis, in the cortical bone, in the layer of calcified cartilage of the epiphysial plate, and in the calcified portion of the articular cartilage next to the supporting bone. Calcification was frequently present in the central portion of the semilunar cartilages.

Alkaline phosphatase was present in the chondrocytes of the hyaline cartilage adjacent to the calcified cartilage in the articular surfaces of the

femur and tibia, patella and semilunar cartilages. In the epiphysial plate there was little or no alkaline phosphatase in the columnar cartilage cells next to the hyaline cartilage, but there was a progressive increase in amount toward the metaphysial end of the column with a maximum amount in the chondrocytes of the hypertrophic and calcified cartilage. There was also heavy activity in the osteoblasts lining the surface of the calcified cartilage, trabeculae of cancellous bone, and endosteal and periosteal surfaces of the cortical bone (Fig. 6).

Although there was a great deal of variation in the degree of staining with toluidine blue, the pattern was consistent. All the hyaline cartilage stained metachromatically, with that of the epiphysial plate staining more intensely than that of the articular cartilage. Calcified cartilage was not metachromatic in undecalcified sections except for faint rims of metachromasia on the inner surfaces of the lacunae of the chondrocytes. After decalcification the calcified cartilage was intensely metachromatic. (Fig. 2). The connective tissue and bone matrix were invariably orthochromatic.

With PAS the hyaline cartilage matrix stained a bright pink, which was not decreased by digestion with diastase. Bone matrix and collagen stained a lighter pink. The chondrocytes of the articular cartilage contained glycogen. Those on the surface contained little glycogen, but the glycogen content of the cells progressively increased passing toward the calcified cartilage with the hypertrophic cells next to the calcified cartilage containing the most. The cytoplasm of the cells of the calcified cartilage was vacuolated and contained little glycogen. The pattern of distribution of glycogen in the chondrocytes of the epiphysial plate was similar; the amount progressively increased to a maximum in the hypertrophic cartilage cells next to the calcified cartilage, but the cells of the calcified cartilage itself contained little or no glycogen.

THE KNEE IN ESTROGEN-TREATED MICE

There was no structural change in the articular cartilages or ligaments of the knees after treatment with estrogen, nor were there any histochemical changes except in association with the formation of new trabeculae of bone.

During the first 5 days of treatment, there was no increase in the number of trabeculae of the metaphysis of the femur or tibia in either sex. Reticular fibers first appeared in the marrow on the 4th or 5th day and were constantly present after the 6th day of treatment. The fibers first appeared projecting into the marrow from the endosteal lining of the cortical bone and from the angles of existing trabeculae in the metaphysis. They formed a network which was dense in its central portion, with fine fibers extending

outward toward the periphery in random fashion (Fig. 7). The growth of fibers inward from the endosteum took place mainly in the upper metaphysis during the early period of treatment and did not reach the diaphysis until the 12th day. With increasing length of treatment there was a progressive increase in the amount of endosteal bone in the metaphysis and a decrease in the number of reticular fibers except at the diaphysis where new growth of endosteal bone and extension of trabeculae into uninvolved marrow was taking place. Reticular fibers always appeared as an outgrowth from the surface of existing bone and never primarily in the marrow. Alkaline phosphatase was present in association with these reticular fibers, but because of the thickness of the sections studied, it was not possible to determine whether or not glycogen and early deposits of calcium salts were also present.

Newly formed trabeculae were randomly oriented in contrast to the trabeculae parallel to the long axis of the bone in untreated mice (Fig. 3). With increasing duration of treatment, the bone extended farther into the metaphysis. By the 12th day, the endosteal bone extended into the diaphysis and after 16 to 18 days the maximum bone growth was reached. Table 1 shows the grading of the amount of endosteal bone and the appearance of reticular fibers in the marrow of the treated animals. Occasional animals failed to show reticular fibers or showed less than the expected amount of endosteal bone growth for a given length of treatment, but the majority conformed to the pattern described above.

DISCUSSION

The general pattern of response to estrogen was similar to that previously described.[5, 6, 10, 16, 17, 18, 21, 23] In untreated animals there was no difference between the staining properties of fibrocartilage, hyaline cartilage, or calcifying cartilage in the knee as compared with the pubic symphysis stained by any of the methods used except for the high glycogen content of the chondrocytes of the symphysial fibrocartilage. However, there were marked histochemical as well as morphological differences between the cartilage in the two sites after treatment with estrogen, in that the symphysis showed increased alkaline phosphatase activity, disappearance of metachromasia, and disappearance of the glycogen from the chondrocytes, while the cartilages of the knee showed no change. Whether the difference in response was due to an intrinsic difference in the cartilage in the two sites which the methods used were not sufficiently sensitive to detect, or whether the difference was due to a hormone secreted locally in response to estrogen,[10, 23] is still open to question.

The earliest changes in the symphysis are a swelling of the cartilage matrix and a proliferation of the cartilage cells of the central fibrocartilage.[25] Crelin[7, 8] observed increased numbers of mitoses in the symphysial fibrocartilage in mice treated with estradiol and relaxin and injected with colchicine 6 hours prior to necropsy. The great number of mitoses occurred in the midline area of the fibrocartilage in the same area which was most highly cellular in the present study. No great amount of mitoses was found in the highly cellular connective tissue adjacent to the eroded margin of the pubes. Although a contributory role of relaxin cannot be excluded, the finding of large numbers of mitoses in areas which were most highly cellular in the estrogen-treated animals suggests that there may also be proliferation of the chondrocytes in these areas in the animals treated with estrogen alone.

Heretofore, it was felt that the greatest contribution to the length of the interpubic ligament was made by proliferation of the highly cellular connective tissue along the margin of the eroded bone of the pubes.[20, 25] The origin of the tissue may be from an inward migration of the cells from the inner layer of the periosteum of the pubes or also at least in part from the marrow by way of channels eroded through the medial margins of the pubes since marrow cells have been observed among the fibroblasts in this tissue.[20, 21, 22] The finding of increased numbers of mitoses and increased cellularity in the central portion of the fibrocartilage suggests that growth of the ligament may take place by proliferation of the cells in this site as well. Why no great number of mitoses was observed in the highly cellular area adjacent to the margins of the pubes remains a perplexing question. A possible source of some of these cells may be liberation of the chondrocytes and osteocytes in areas of erosion of bone and cartilage,[11, 20, 22] or erosion of bone and dissolution of the cartilage matrix may permit the dorsal and ventral portions of the articular capsule to approach one another along the margin of the eroded bone and thus create the appearance of increased cellularity through a merging of the highly cellular portions of the capsule.[9]

The swelling of the ground substance of the cartilage which was observed might be explained on the basis of an uptake of water as suggested by Talmage[27, 28] and Hall.[28] A change in the refractive index of the ground substance as a result of water uptake may be responsible for the glassy, homogeneous appearance of the matrix of the hyalinized cartilage by masking the collagen fibers. The suggestion of depolymerization of the glycoproteins of the ground substance[29] remains an attractive one in view of the apparent disappearance of the ground substance in the transition between cartilage and connective tissue. Since continuous transitions were observed

between cartilage and connective tissue, it appears that much of the cellular and extracellular constituents of the interpubic ligaments are derived from the symphysial cartilage by dissolution of the ground substance.

The results obtained with the Gomori method for alkaline phosphatase tend to confirm the suggestion of other workers[25, 31, 37, 38] that the increase in alkaline phosphatase activity in the pubic symphysis after treatment with estrogen parallels the development of rapidly proliferating connective tissue. Activity was high in the central part of the symphysis where cellular proliferation occurred in the treated animals and absent in this area in untreated animals. Activity was also high along the margins of the eroded bone where many reticular fibers were present. The presence of alkaline phosphatase in the fibrocartilage adjacent to the hyaline cartilage caps of the pubes in both untreated and treated animals was related to the development of islands of calcified cartilage within the fibrocartilage and therefore an example of the well-known association of alkaline phosphatase with chondrocytes in the process of calcification. The role of the alkaline phosphatase, other than its association with proliferating connective tissue, is not yet clear.

Metachromatic staining of cartilage is largely due to the presence of chondroitin sulfuric acid in the ground substance.[13, 39] The disappearance of metachromasia from the symphysis pubis after treatment with estrogen has not been conclusively explained. Loss of metachromasia is probably due to a depolymerization of the chondroitin sulfuric acid of the ground substance, since metachromasia is dependent upon a high molecular weight as well as upon the sulfuric acid group. However, another possible explanation for the loss of metachromasia is that the reactive groups of the chondroitin sulfuric acid may be bound by another ion in competition with the dye. The competitive effect of calcium ion has been demonstrated,[25, 32] and there is an increase in the calcium content of the symphysial tissue after treatment with estrogen.[38] However, if calcium were involved, one might expect a return of metachromasia after decalcification, which is not the case. French and Benditt[13] showed that the presence of basic proteins may mask the metachromatic staining of mucopolysaccharides by toluidine blue and imitate the effect of hyaluronidase or chondroitinase in eliminating metachromatic staining of chondroitin sulfuric acid *in vitro*. That some change in proteins does occur in the symphysis after treatment with estrogen is evident by the work of Frieden and Martin[15] and Frieden,[14] who showed that there was an increase in the incorporation of labelled glycine by the symphysis pubis of castrate guinea pigs treated with estradiol. Quantitative studies of total chondroitin sulfuric acid content of the symphysial tissue before and after

treatment with estrogen would be of considerable value in determining whether disappearance of metachromasia is due to depolymerization of the chondroitin sulfuric acid or to masking of its staining by protein or an ion such as calcium.

The irregular staining of the symphysis of the untreated animals with toluidine blue remains unexplained. It has been observed by other workers.[9] Wislocki, Singer, and Dempsey[41] reported that thin sections are necessary for optimal demonstration of metachromasia since thicker sections tend to appear orthochromatic. Part of the difficulty in the present experiment may have been due to the thickness of the sections and to variations in thickness due to variable expansion after application of melted paraffin to the surface of the block at the time of sectioning.

The PAS staining of the cartilage of the symphysis did not parallel the staining with toluidine blue, since there was no decrease in the intensity of staining of the matrix at any time during the period of treatment with estrogen. Hall[23] found a diminution in the intensity of PAS staining as well as metachromasia in the symphysis during the last 5 days of pregnancy in the mouse. The difference between her findings and those of the present experiment are probably related to the presence of relaxin, since Perl and Catchpole[30] observed a decrease in the intensity of PAS staining of the symphysis of the guinea pig after treatment with estrogen plus relaxin but not after estrogen alone. Depolymerization of the ground substance might be expected to release additional reactive groups and increase the intensity of staining with PAS; however, uptake of water and dissolution of the matrix might be expected to decrease the staining intensity. The differences between the effects of estrogen alone and of estrogen plus relaxin may be related to differences in the equilibrium attained between the two processes. The significance of the disappearance of glycogen from the chondrocytes of the symphysis in response to estrogen is as yet unclear. The absence of glycogen in some of the sections from untreated animals may have been due to artefact since those sections which did not contain glycogen in the chondrocytes of the central fibrocartilage also failed to show the usual amount of glycogen in the chondrocytes of the hypertrophic cartilage.

Growth of medullary bone at the knee appears to occur primarily at the ends of existing trabeculae and on the endosteal surface of cortical bone.[33, 34, 35, 39, 40] The process begins by extension of reticular fibers from the ends of trabeculae and from the endosteal surface of the cortical bone in the metaphysis. These gradually increase in number to form a network with collagen fibers in its central portion. Alkaline phosphatase appears in association with these fibers. It was not possible in the present study to

observe an increase in the PAS staining around the reticular fibers or in the adjacent cells, or to detect fine granules of calcium salts among the fibers in intermediate states between reticular fibers and fully calcified trabeculae. Such findings might be expected in view of their presence in the development of membranous bone.[2] It is possible that the absence of such findings in the present experiment was apparent only because of the disruption of the tissue as a result of sectioning without decalcification and to the obscuring of fine detail by the thickness of the sections.

The reticular fibers seem definitely to be a preliminary step in the growth of new bone. They appeared slightly before any increase in the number or size of the bony trabeculae became evident and persisted during the time in which bone growth was taking place with a tendency to decrease in number toward the end of the experiment when maximum bone formation had already taken place. The growth of endosteal bone appears to be similar to the formation of membranous bone rather than to the simple addition of new bone along the surface of existing trabeculae by the osteoblasts. The origin of the osteogenic cells which lay down the bone in the aggregates of reticular fibers was not determined. They may develop from the primitive reticular cells of the marrow as suggested by Urist, Budy, and McLean[39] or they may migrate outward from existing trabeculae.

SUMMARY

Observations using histochemical methods were made of the symphysis pubis and of the knee joint in castrate male and female mice at daily intervals during a 30-day period of treatment with estrogen. A marked increase in flexibility of the symphysis was evident after 6 days of treatment in females and after 11 days in males. Flexibility determined by manual manipulation did not increase after 19 days of treatment in either sex. There was a swelling of the matrix and proliferation of the chondrocytes in the central fibrocartilage of the symphysis in estrogen-treated mice of both sexes. Glycogen disappeared from the chondrocytes of the fibrocartilage after the 5th day in females and after the 9th day in males. Metachromasia disappeared from the fibrocartilage matrix after the 9th day in females and after the 14th day in males. Alkaline phosphatase was present in the symphysial fibrocartilage after 3 days of treatment in both sexes. There was resorption of the medial margins of the pubes beginning at the craniodorsal end of the symphysis on the 6th day of treatment in both sexes. After 30 days of treatment the interpubic tissue was composed largely of connective tissue in which a few islands of hyalinized cartilage remained.

The knees of the estrogen-treated mice stained similarly to those of the untreated mice. After 4 to 6 days of treatment reticular fibers were present in the bone marrow near the ends of trabeculae and along the endosteum of cortical bone. The number of trabeculae within the epiphysis and metaphysis increased with increasing length of estrogen treatment so that after 16 days the marrow was filled with a network of cancellous bone. The trabeculae in the estrogen-treated animals were thicker than those in the controls and were oriented in random fashion rather than parallel to the long axis of the bone.

ACKNOWLEDGMENT

I wish to thank Dr. E. S. Crelin of the Department of Anatomy, Yale University School of Medicine, who directed the research here reported, for his generous support and many helpful suggestions and criticisms during the course of the experiment and preparation of the manuscript.

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A STUDY OF THE ERUPTION RATE OF THE RAT MANDIBULAR INCISOR†‡

INTRODUCTION

Several different theories have been advanced to explain the mechanism of tooth eruption. This has been defined by Massler and Schour[21] as the process whereby a developing tooth migrates from an intraosseous location in the jaw to its functional position within the oral cavity. They also evaluated each of these theories with regard to clinical and experimental observations. Substantial factual evidence for the support of any one of them is lacking, since this aspect of tooth development has been little investigated. However, much support has been given to a concept linking the vascularity of the pulpal and periodontal tissues with tooth eruption and theoretically suggesting that the local eruptive force results from osmotic pressure or tissue tension.

Constant[9] was the first to suggest a relationship between vascularity and eruption. He considered the blood pressure exerted in the vascular tissues at the base of a forming tooth as the active mechanical factor in eruption. Tomes[31] agreed that the blood pressure, by keeping up a state of general tension in the area, could drive a tooth in any direction. The rich vascular bed of the rat incisor was believed by Boyle[5] to be comparable in effect to a hydraulic chamber. Schour and Massler[25] concluded that the eruptive force was probably related to vascularity and tissue tension at the basal end of the tooth.

Several investigators (Leist,[20] Breitner and Leist,[4] King, [19] and Edwards and Kitchin[11]) by performing unilateral cervical sympathectomies in guinea pigs and rabbits induced temporary accelerations in eruption rates;¶ and,

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† Presented in part at the 35th General Meeting of the International Association of Dental Research, Atlantic City, New Jersey, March 21-24, 1957.

‡ Research supported by a U.S. Public Health Service grant from the National Institutes of Health, National Institute of Dental Research, Department of Health, Education and Welfare, Bethesda, Maryland.

¶ Eruption rate refers to the speed of eruption, in particular that of the continuously growing incisors of rodents. This rate can easily be determined by marking the tooth and measuring at a later time the distance that the mark has migrated from a fixed point.

Received for publication May 31, 1957.

in certain cases (kittens[11]), precocious eruption* was achieved. All concluded that this was due to vasodilation in the jaws on the operated side, since the vasoconstrictor fibers had been severed. However, only King[19] claimed that he could histologically demonstrate increased vascularity in the dental and periodontal tissues. Butcher and Taylor[7] failed to increase the eruption rate of the rat incisor by cervical sympathectomy. But their operative procedure differed from that of those cited above since they also "transected" the common carotid.

TABLE 1. EXPERIMENTAL ARRANGEMENT

GROUP	SUBGROUP	CONTROL PERIOD 1 WEEK	TREATMENT PERIOD 3-4 WEEKS 1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK
A	I		PRISCOLINE HYDROCHLORIDE 3.57mg/cc			
	II	PERIOD OF	"	"	"	NO TREATMENT
B	I	NO TREATMENT	LEVOPHED BITARTRATE .667mg/cc			
	II	TO DETERMINE	"	"	"	NO TREATMENT
C	I	THE NORMAL	DISTILLED		WATER	
	II	WEEKLY	"		"	NO TREATMENT
D	I	ERUPTION	NEEDLE		INJECTION	
	II	RATE FOR	"		"	NO TREATMENT
E	I	ALL ANIMALS	NO TREATMENT		HANDLED ANIMALS	
	II		"	"	"	NO TREATMENT

That a relationship exists between endocrines and tooth eruption was suggested by Keith[18] as early as 1911. Subsequent experimentation with injections of acetyl thyroxin (Hoskins[16]) and thyroxin (Karnofsky and Cronkite[17]) caused precocious eruption of the incisor teeth in newborn rats. Normal rats similarly treated with thyroxin (Herzberg and Schour cited by Schour and Massler[25]) had accelerated eruption rates. Hypophysectomy,[4, 20, 27] thyroidectomy,[3, 22] and adrenalectomy[18, 29] are all capable of retarding the eruption rate of the rat incisor. Nevertheless, the actual mechanism by which endocrines influence tooth eruption has not yet been fully explained. Hunter (cited by Keith[18]) recognized an active formation of osseous vessels

* Precocious eruption means the earlier than expected appearance of any tooth within the oral cavity and is used as a measure of eruption speed in many young experimental animals.

concomitant with growth of bone in acromegaly, ". . . capillaries and especially the venous sinuses, increase in number and size." The converse of this observation was born out in hypophysectomized[4, 26, 27] and thyroidectomized[8] rats, when a marked reduction in vascularity of pulpal and periodontal tissues was correlated with retardation of eruption. Replacement therapy with growth hormone and thyroxin, separately and together, progressively returned both vascularity and eruption rates towards normal.

The above hypotheses and experimental observations suggest that eruption of teeth is dependent upon the dental vascular supply. Conclusive

TABLE 2. INJECTION PROCEDURE

GROUP	SUBGROUP	INJECTED AGENT	DILUTING AGENT	VOL. INJ.	DRUG CONC.	NEEDLE GAUGE	NO. INJ.	SITE OF INJECTIONS
A	I	PRISCOLINE	DISTILLED	.08cc	.29mg/.08cc	25g	28	EXTRAORAL—
	II	HYDROCHLORIDE	WATER	"	"	"	21	THROUGH
B	I	LEVOPHED	5% DEXTROSE	"	.05mg/.08cc	"	28	THE RIGHT
	II	BITARTRATE	DIS. WATER	"	"	"	21	MASSETER MUSCLE
C	I	DISTILLED	—	"	—	"	28	TO THE BASE
	II	WATER	—	"	—	"	21	OF THE
D	I	NONE	—	—	—	"	28	MANDIBULAR
	II	"	—	—	—	"	21	INCISOR

evidence of this relationship and how a local driving force culminates from its presence is lacking. In view of this, a study was initiated to determine whether the eruption rate of the right mandibular rat incisor could be altered by local injections of a vasodilating and a vasoconstricting drug. The immediate aim was to encourage local changes in the vascular bed of the treated incisor while at the same time minimizing, if possible, systemic manifestations. This investigator felt that an experiment of such a nature might clarify the basis for a vascular theory of eruption and shed further light on this physiological phenomenon.

MATERIALS AND METHODS

For this study 120 albino rats (Sprague-Dawley strain) approximately four months old were used. Separated as to sex and placed on a Purina rat chow diet for two weeks they were then divided into five equal groups of 24 animals (12 males and 12 females). Each rat was anesthetized in an ether jar, weighed, and had its right mandibular incisor notched with a fine jeweler's file at the labio-gingival margin. This procedure, plus measuring with a vernier caliper the distance that the notch had migrated from the gum line was repeated every seventh day until the experiment was completed. After

a one-week control period, treatment of the first four groups was started and consisted of the following daily injections (Tables 1 and 2).

Group A received the peripheral vasodilator, Priscoline hydrochloride, diluted to 3.57 mg./cc. with distilled water.

Group B was injected with the vasoconstrictor, Levophed bitartrate,[12] .667 mg./cc. in 5 per cent dextrose distilled water.

Group C received distilled water injections.

Group D merely had daily needle injections.

TABLE 3. ANIMAL RECORD. ALBINO RATS (SPRAGUE-DAWLEY STRAIN) APPROXIMATELY 120 DAYS OLD

GROUP	SUBGROUP	START	♂	♀	FINISH*	♂	♀	HISTOLOGY	♂	♀
A	I	24	12	6	22	11	5	14	7	4
				12			11			7
	II		12	6		11	6		7	3
				12			11			7
B	I	24	12	6	21	10	4	15	8	5
				12			10			8
	II		12	6		11	6		7	3
				12			11			7
C	I	24	12	6	20	10	4	16	8	5
				12			10			8
	II		12	6		10	6		8	3
				12			10			8
D	I	24	12	6	20	10	4	16	8	5
				12			9			9
	II		12	6		10	5		8	4
				12			10			8
E	I	24	12	6	20	10	5	16	8	4
				12			11			7
	II		12	6		10	6		8	3
				12			10			8
TOTAL		120	60	60	103	51	52	77	39	38

*STATISTICAL ANALYSIS IS BASED ON ANIMALS LISTED IN THIS COLUMN

All injections were administered with a 25-gauge needle at the base of the right mandibular incisor by means of an extra-oral approach through the masseter muscle. In the cases of Groups A, B, and C the volume of injected fluid was .08 cc. Daily injections were given for 21 days and then each group was equally divided into subgroups I and II. Subgroup I had its treatment continued during the final week, while the injections were stopped in subgroup II.

Group E served as untreated controls for a total of three weeks. Starting on the 21st day these animals were handled once a day by holding each rat for 10 seconds as though a mandibular injection were to be given. During the final week of experimentation this group was also divided, with handling being continued in subgroup I.

A few animals were sacrificed weekly for histological evaluation and additional ones at the end of the experiment (Table 3). Sacrifice was by chloroform inhalation one-half hour after the last treatment. Tissues including mandibles and vital organs were recovered, examined grossly, fixed in formalin, decalcified when necessary, and stained with hematoxylin-eosin.

Untreated reserve rats with eruption rates of 2.8 mm./week and starved animals whose eruption rates measured 2.1 mm./week were also sacrificed for histological comparisons with the treated groups.

Statistical analyses of weekly weight gains and eruption rates were carried out.

RESULTS

No animals, when examined grossly during the experiment, exhibited adverse effects from the treatment. Evidence of infection at the injection

TABLE 4. WEEKLY ERUPTION RATE AND STANDARD DEVIATION FOR MANDIBULAR INCISOR

GROUP	NUMBER OF RATS	WEEKLY ERUPTION RATE AND STANDARD DEVIATION			
		CONTROL WEEK	TREATMENT PERIOD 3 WEEKS		
			1ST WEEK	2ND WEEK	3RD WEEK
A PRISCOLINE	22	2.82 ±0.09	3.28 ±0.39	3.38 ±0.29	3.31 ±0.31
B LEVOPHED	21	2.79 ±0.13	2.76 ±0.32	2.99 ±0.20	3.21 ±0.33
C DIST. WATER	20	2.81 ±0.19	3.27 ±0.29	3.55 ±0.34	3.09 ±0.29
D NEEDLE INJ.	20	2.82 ±0.29	3.38 ±0.55	3.44 ±0.58	3.40 ±0.36
E CONTROLS	20	2.79 ±0.14	2.80 ±0.24	2.96 ±0.41	3.46* ±0.37
COMBINED GROUPS	120	2.80** ±0.17	—	—	—

* NO TREATMENT UNTIL END OF 2ND WEEK. RATS HANDLED DAILY DURING 3RD WEEK.
** REPRESENTS ORIGINAL 120 RATS TO DETERMINE NORMAL WEEKLY ERUPTION RATE.

site or loss of weight were lacking. Rats reacted similarly to daily handling and injections as well as to weekly ether anesthesia by becoming excited at these times.

Eruption rates for mandibular incisors averaged 2.8 mm./week (S.D. = ± 0.17 mm.) when measured during the control period. The Priscoline, distilled water, and needle-injected groups experienced immediate accelerations in eruption rate reaching their respective peaks after two weeks of treatment. Rats receiving the vasoconstrictor, Levophed bitartrate, exhibited near normal eruption rates for one week before a gradual increase set in. Controls showed a slight increase in eruption rate during the last week before handling. But a sudden and significant increase was apparent once this procedure was initiated (Table 4 and Fig. 1).

The left mandibular incisors at the end of the third week of the treatment period measured the same as the right.

Except for the Priscoline-treated animals, there was no significant difference in the respective eruption rates when treatment was stopped in one-half the animals. Subgroup AI, with continued vasodilator injections, showed a further slight increase in eruption rate to 3.4 mm., while subgroup AII exhibited a sudden drop to 2.88 mm. when treatment was stopped (Fig. 2). Cessation of handling during the last week in subgroup E did not alter the increased eruption rate in these rats.

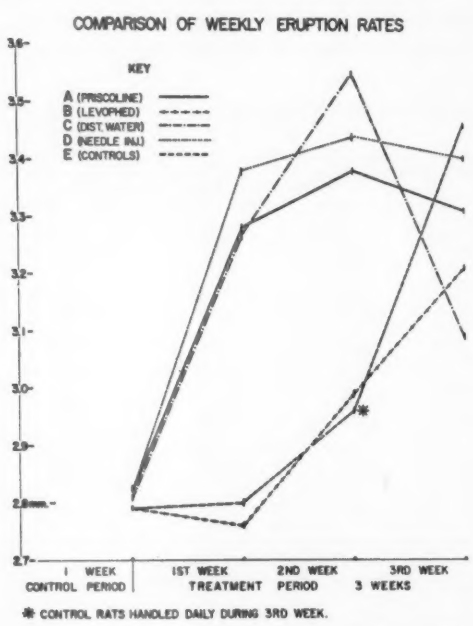


FIG. 1. Shows the alterations in weekly eruption rates. Note: groups A, C, and D reached their respective peaks after two weeks of treatment; group B showed an increase in eruption rate after one week of no response over normal; group E exhibited a sudden increase in eruption rate when handled.

No correlation could be made between increased eruption rates, weight gains, sexes, or the treatments given. Males and females gained an average of 15 and 8 gms./week respectively. Weight gains were greatest during the first week of the investigation and subsequently decreased in a linear curve as the experiment continued.

When jaws of rats with elevated, normal, and subnormal eruption rates were dissected, cleaned, and compared grossly, marked differences were apparent at the basal or formative end of the continuously growing incisors. In the normal untreated rat with a weekly eruption rate of 2.8 mm./week this area stands out on the buccal aspect of the mandible as a rounded projection, directed upwards and backwards beneath the coronoid process. Its posterior border is below but even with the anterior margin of the mandibular notch. In rats with elevated eruption rates (3.6 mm. and up) the projection is more prominent buccally and extends higher and further distally than the normal. These findings were directly proportional to an increase in eruption rate. The converse was apparent in rats with below

normal eruption rates (2.1 mm./week), for they showed only a slight buccal projection that did not extend upwards and backwards as much as in the normal animals (Figs. 3 and 4). These findings were further substantiated when ground jaw sections were prepared.

Histological distinctions based upon the type of drug or treatment given were not significant. Regardless of treatment there was a correlation

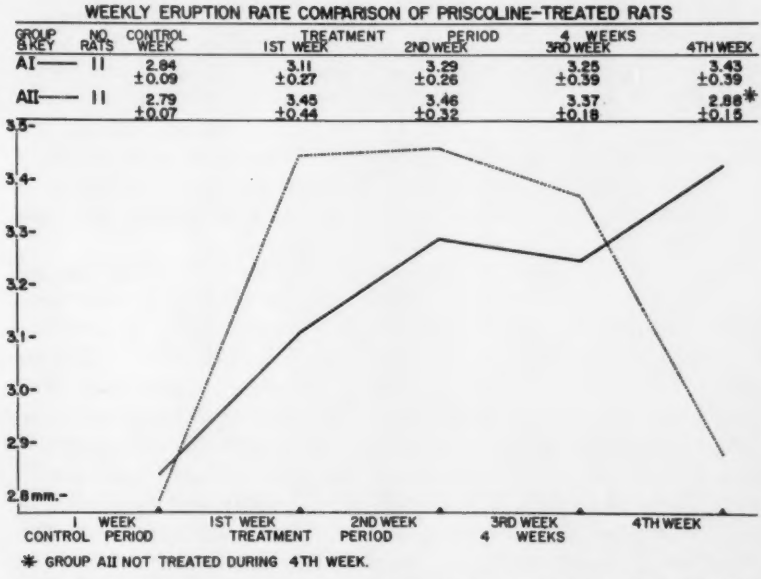


FIG. 2. Illustrates the return to a normal eruption rate one week after Priscoline treatment was discontinued in subgroup AII.

between incisor eruption rates and microscopic findings. In those animals with elevated eruption rates these were:

1. An increase in vascularity about the tooth, characterized by an enlargement of capillaries and vascular spaces and an increased volume of tissue fluid on both the periodontal and enamel sides of the incisor, noted when there was an acceleration in eruption rate.

2. The alveolar bone had a scalloped-out appearance adjacent to the base of the incisor and frequently exhibited microscopic fractures along the periodontal membrane attachments. At these times periodontal attachments appeared narrower and their fibers were stretched.

3. Enamel calcification was poorest and predentin thickest in these instances.

4. Organ study did not reveal significant differences except possibly in the thyroid and adrenals. When eruption rates were exceptionally high (4.2-4.5 mm./week), thyroid follicles often appeared larger and their colloidal material stained deeper. The adrenals were smaller with capsular infoldings in rats with subnormal eruption rates (2.1 mm./week).

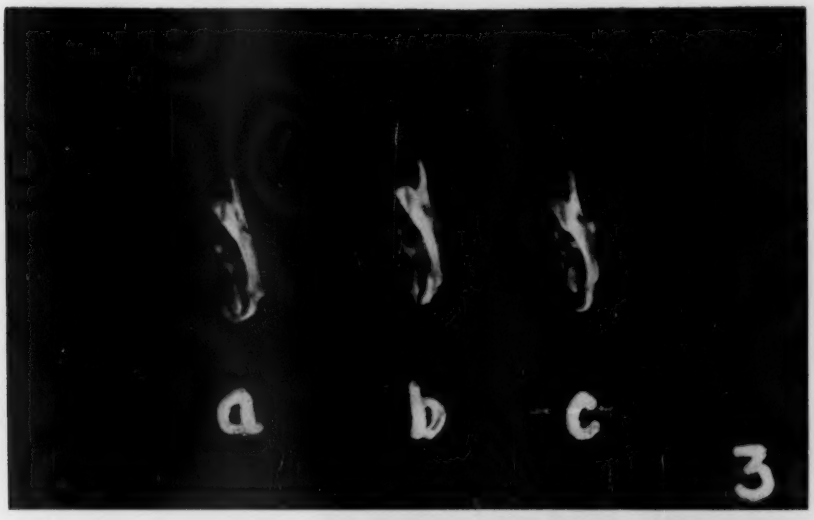
DISCUSSION

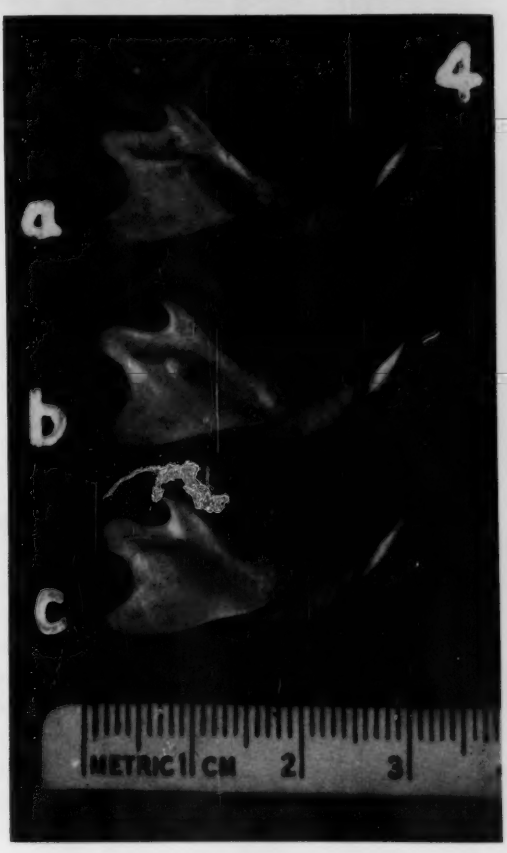
The normal extrusive growth rate of the adult albino rat mandibular incisor has been measured to be 2.8 mm./week (Addison and Appleton[1]), 2.78 mm./week (Downs[10]), and 2.9 mm./week (Shadle, Wagner, and Jacobs[28]). This investigator's finding of 2.80 mm./week with a S.D. of $\pm$ 0.17 as computed in 120 young adult Sprague-Dawley rats of both sexes when measured over a one-week control period is in keeping with these former results.

Maintaining treatments locally to the base of the right incisor was not possible. This was evidenced by the fact that the left incisor, when measured for extrusive growth, mimicked its right counterpart. Nevertheless, the results obtained were significant. A reaction which will be called the "excitability factor" may have caused the increased eruption rate. This became apparent in the untreated control animals when they were subjected to daily handling in addition to weekly ether anesthesia. Defecation and urination as measures of emotionality in rats have been used in the past.[9,14] The behavior of rats when given mandibular injections, roughly handled, or subjected to ether anesthesia was consistent in that defecation and urination were markedly increased at these times. This may well be one of the overt signs of the "excitability factor's" presence.

An accelerated eruption rate due to rough handling has not previously been reported in the literature. Knowledge of its superimposition upon the treatments given in this experiment obscures any interpretation of those treatments and may present a factor heretofore not taken into account in other eruption rate studies where daily handling was a part of the procedure.

It is not clear how this "excitability factor" affects eruption rate. It is assumed to be a systemic manifestation which may be acting (i) on the attritional activity of the incisors; (ii) directly on the eruptive force within the jaws, or (iii) a combination of both. In studying the eruption rate of the rat incisor, attrition must be taken into consideration. Presumably, the eruptive potential is regulated by attrition and must always be greater than





FIGS. 3 AND 4. Gross comparison of right mandibles to illustrate the position of the formative dental organ. Posterior view (Fig. 3). Lateral view of same specimens (Fig. 4). *Specimen a:* subnormal eruption rate (2.1 mm./wk.), note almost no buccal projection; forward and downward shift of basal or growing end of incisor; *specimen b:* normal eruption rate (2.8 mm./wk.); *specimen c:* accelerated eruption rate (3.9 mm./wk.), note enlarged buccal projection of the formative dental organ which has migrated upwards and backwards.

its expression in normal animals (Hoffman cited by Schour and Massler,[21] Taylor and Butcher,[30] and Orsós and Bartha[24]).

The increased local vascularity and tissue fluid content which were apparent whenever there was an accelerated eruption rate indicates a correlation between the dental vascular bed and eruption. But this does not prove that vascularity serves as the eruptive force within the jaws. When the behavior of the growth center of the incisor is taken into consideration, it appears that vascularity merely serves as a local control mechanism over the eruptive force. If the activity of the odontogenic epithelium and pulpal connective tissue were not stimulated as an accelerated eruption rate is achieved, the position of the dental organ would be shifted anteriorly, and eventually the incisor would be extruded from the jaw. However, this forward shift is only noticeable when there is a decrease in eruption rate. With eruption rates that are exceptionally great (3.8 mm.-4.5 mm./week), there is displacement of the odontogenic epithelium in a posterior superior lateral direction with concomitant bone resorption in these areas. In other words the tooth is actually elongating faster than it is erupting in a forward direction. This phenomena indicates that excessive attrition is not here the primary cause of the increased eruption rate. It appears rather that there is an increase in attrition but still not enough to allow full expression of the increased eruption rate.

Baume *et al.*[5] also recognized that the odontogenic epithelial diaphragm was not fixed in its position. When delayed eruption of the rat incisor resulted from x-ray irradiation, Dale[9] noted a concomitant cessation of both amelogenesis and dentinogenesis. Medak *et al.*[22, 23] in similar studies attributed a deceleration of eruption to the primary destruction of the fast growing basal end of the pulpal connective tissue. The association of vascularity with amelogenesis and dentinogenesis as well as a relationship with eruption has been noted by Schour and VanDyke in the hypophysectomized rat[26, 27] and by Baume *et al.* in thyroidectomy[3] and hypophysectomy[4] studies. Removal of the pulp and Hertwig's sheath (Herzberg and Schour[15]), however, did not alter the normal eruption rate of the rat incisor still under attritional pressure. Apparently this eliminated any role of the pulp and the formative dental organ in the eruptive process of continuously growing teeth. However, normal conditions within the jaw were altered by surgery and the state of the tooth then paralleled that of an "erupting" root fragment or bone spicule activated by pressure on the overlying tissue.

Interpreting the actions of the vasodilating and vasoconstricting drugs in this experiment was difficult since the effect of the "excitability factor," which alone may increase eruption rate, was superimposed. When only the

Priscoline-treated group showed a drop to a normal eruption rate within one week after discontinuing treatment, it indicated that Priscoline did exercise an effect. By the same token Levophed, with its ability to maintain a near normal eruption rate for one week despite the "excitability factor," exhibited that it too had some influence.

SUMMARY AND CONCLUSIONS

The influence of local injections of Priscoline hydrochloride and Levophed bitartrate as well as daily handling upon the eruption rate of the mandibular incisor in 120 albino rats (Sprague-Dawley) was studied. Normal and sham-treated controls together with rats exhibiting subnormal eruption rates were compared grossly and histologically with experimental groups. A statistical analysis, based upon weekly weight gains and eruption rate determinations, was computed.

This investigation showed that:

1. The normal eruption rate for the mandibular incisor of Sprague-Dawley rats, 120-150 days old, was found to be 2.8 mm./week.

2. An "excitability factor" aroused by weekly ether anesthesia and daily handling caused an increased eruption rate in all groups. This factor which is discussed may be a peculiarity of the Sprague-Dawley strain or rats in general.

3. The gross and histological findings could be correlated with variations in eruption rate but not with the treatments given.

4. Increases in vascularity and tissue fluid content were directly correlated with accelerated eruption rates which sometimes reached 4.5 mm./week.

5. When the eruption rate was substantially increased, there was a backward and upward shifting of the odontogenic growth center of the incisor. A forward and downward shifting of this area in cases of subnormal eruption was also noted.

6. Eruption of the continuously growing rat incisor is locally controlled by vascularity and attrition, while the eruptive force is probably derived from the activity of the proliferating odontogenic epithelium and pulpal cells.

ACKNOWLEDGMENT

I acknowledge with thanks the valuable advice and assistance given this study by Dr. Harold S. Fleming, Dr. H. S. N. Greene for the use of the department's facilities, and Dr. Colin White for his aid in preparing the statistical analyses.

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WOODWARD LECTURE

September 30, 1957

PROPRIOCEPTION IN INSECTS. By O. E. Lowenstein, Professor of Zoology and Physiology, University of Birmingham, England.

Proprioceptors strikingly similar to the muscle spindles of vertebrates and the abdominal stretch receptors of crabs and lobsters have recently been found in the abdomen of the giant silk moth, *Cecropia*. A single sensory neuron, whose cell body lies peripherally against the special muscle concerned, sends its axon centrally, lying among the motor innervation. The individual organ can be isolated and mounted on a device for administering a measurable stretch. Sensory output of the organ varies linearly with the amount of stretch up to a maximum when the discharge decreases. Morphologically, similar organs are found in Orthoptera but the association with a muscle is looser and the organ may even lie diagonally across a muscle. In the cockroach, the organ is found in the dorsal musculature associated with connective tissue. Similar organs have been demonstrated in the second and third thoracic segments of the stick insect and present evidence indicates that they may also be found in the thorax of the moth. The orthopteran electrophysiological picture is the same as that in the moth. In the honey bee, the organ runs diagonally across muscle fibers and connective tissue associated with it apparently is a hollow tube. Three systems are found in Odonata nymphs: a longitudinal one, a dorsal-ventral one, and a diagonal one. The last is morphologically different from the other two in that it contains three neural cell bodies and may be associated with the respiratory movements of the animal, whereas the other two relay stresses in longitudinal body movements such as propulsion and bending. Sensory output has been recorded from the longitudinal and dorsal-ventral organs but not so far from the oblique ones.

A possible phylogenetic development is suggested from the generalized organ of the Odonata, in which a discrete connective tissue organ generates the sensory output, to the higher Lepidoptera, in which the sensory organ is integrated into the muscles which are under proprioceptive control.

G. E. W. 3RD

YALE MEDICAL SOCIETY LECTURE

October 14, 1957

EXPERIMENTAL HYPERSENSITIVITY AND HUMAN DISEASE. By Frederick G. Germuth, Jr., Associate Professor of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Experimental serum sickness disease in animals provides a model type of anaphylactic reaction which in certain respects appears to resemble in its pathology the human lesion of periarteritis nodosa. Injection of crystalline bovine albumin into rabbits gives a different curve of antigen titer with respect to time than does injection of homologous protein. From about day 8 the rate of clearance of heterologous protein is the more rapid, and it is suggested that this effect is due to neutralization by rising antibody. Serial sacrifices demonstrate that lesions appear between about day 8 and

day 18 with a peak incidence at day 13. It seems that the animals must produce a certain amount of antibody while antigen is still present for lesions to develop. Passive transfer experiments bear out this conclusion.

Various experimenters have compared some of the resulting lesions to those of post-streptococcal diseases; however, the resulting glomerular and cardiac lesions more closely resemble those found in human periarteritis nodosa. With repeated and prolonged injections both the necrotizing and the granulomatous types of renal arteritis appear. The types and locations of lesions depend upon the duration of sensitization and correlate well with the lesions of periarteritis.

It has long been known that in the presence of excess antigen, soluble antigen-antibody complexes are formed. On this basis it is proposed that both antigen and antibody are carried to the receptor organ together, rather than one being fixed independently of the other. Experimentally the soluble complex is capable of producing shock in the guinea pig and the Arthus reaction and serum sickness type lesions in the rabbit. It has been shown that this is not due to transferred histamine or proteolytic enzyme. An *in vivo* Oudin test is being developed to investigate further the mechanism of lesion formation.

N. R. M.

WOODWARD LECTURE

October 23, 1957

EARLY MAMMALS. By Kenneth A. Kermack, University College, London, England.

Numerous tiny fossils of late Triassic or early Jurassic Age which were recently found in fissures in a quarry in South Wales show both mammalian and reptilian characters. The tricuspid teeth are fairly close to those of the Tricodonta and the jaw articulation is between the squamosal and the dentary bones; both are good mammalian characters. The lower jaw bone is similar to that in *Docodon,* an extinct genus grouped by Simpson in the mammalian order Pantotheria. They both show a trough at the posterior margin of the dentary. The same trough is found in *Tritylodon,* a perfectly good reptile, where the function is known to be that of lodging the articular bone. Therefore both the new Welsh animals and *Docodon* had a double jaw articulation—one reptilian, the other mammalian. They should both remain for the present in the Mammalia, however, since they have mammalian teeth and because the main jaw articulation is mammalian. In addition, they share a remarkable, and apparently nonadaptive, character with the monotremes, which are regarded as a group of true mammals. In both, the petrosal bone surrounding the inner ear is penetrated by the mandibular branch of the fifth cranial nerve while in all other mammals this nerve passes through the alisphenoid.

Thus, either this specialization of the petrosal has arisen quite independently in the Docodonta and in the monotremes, or the latter group must have evolved from the reptiles independently of the other mammals.

G. E. W. 3RD

MEDICAL ETHICS. A GUIDE TO STUDENTS AND PRACTITIONERS. Maurice Davidson, Ed. London, Lloyd-Luke (Medical Books) Ltd., 1957. 165 pp. 20 shillings.

The editor, believing that, "the trend of present-day medical teaching is in the direction of developing the theoretical at the expense of the practical," has compiled a book containing chapters by himself and eleven other eminent contributors which deal "with the doctor's general outlook on his professional life, and with all sorts of considerations, which, though little or nothing is said about them in the routine course of his training, may nevertheless have a profound influence upon his future career, and upon the success, in the best sense of the word, which he is likely to attain in the general handling of his patients." Such items as professional etiquette between physicians, the care of the dying, the doctor's duty to his church and his family, certain medical-legal problems, the ethical basis of experimental medicine, and the history of the Hippocratic oath are considered.

The result is a good book, reading of which would be rewarding to any physician. Some of the chapters, such as the ones dealing with the General Medical Council of the United Kingdom and medical-legal matters, are not wholly applicable to practice in other countries. Also, the discussions of relations between family doctors and consultants are not entirely suited to practice in the United States where there seems to be less of a gap between the two kinds of practitioners than in Great Britain.

Items of medical etiquette, such as avoidance of advertising, avoidance of public criticism of colleagues, the procedure for calling in a consultant, decision to carry out therapeutic abortions, etc., are dealt with in satisfactory fashion. The chapter on the doctor's responsibility to his family takes up only the liability of overlooking disease within his own household, but does not touch on the subtler and really more serious problem: how the busy doctor is to be a good husband and father.

The chapters by Davidson and Batten, on 'What to Tell the Gravely Ill Patient,' and 'Management of the Hopeless Case,' are outstanding. They overlap a little but are thoughtful, generally in agreement, and obviously based on wide experience. Here is an excerpt from Batten:

> . . . nothing can relieve the doctor of trying to decide what will be best for *this* patient in *this* case. After all, the patient asking for the truth knows not what he asks. The doctor does at least know what the patient must hear if the truth is told to him. He must judge, with whatever help he can get, how much of the whole truth he can give without adding to the wounds already dealt by the disease a shattering blow to that very fortitude by which the patient must now live.

The chapter by Hoyle, on 'Care of the Dying,' is largely a pharmacological treatise on narcotics, and is really summarized in one good sentence: "The occasions when morphine should be withheld do not concern the dying." The chapter also contains this startling passage: "If there are occasions when hastening death seems the most sensible and humane procedure, the proper course then becomes a matter for one's own conscience, a guide to conduct is found by regarding the circumstances as affecting a close personal relative. The first essential on these occasions is to keep one's own counsel. Nothing should ever be done at the request of third parties.

As for the means, morphine in quickly cumulative doses is one of the best. Insulin produces unconsciousness easily; as little as 40 units may be fatal to a cachectic patient, while others need much more. Venesection is unreliable. Relatives should be warned that the patient is worse, and is reaching unconsciousness rapidly. One other point about such treatment—the doctor should always give the drug himself, for he takes the direct responsibility in that way."

The book is easy and pleasing to read, and unique in its coverage of important and neglected subjects.

PAUL B. BEESON

SIGNS AND SYMPTOMS: APPLIED PATHOLOGIC PHYSIOLOGY AND CLINICAL INTERPRETATION. Cyril Mitchell MacBride, Ed. 3d. ed. Philadelphia, J. B. Lippincott, 1957. xviii, 973 pp. $12.00.

In this valuable work the cardinal manifestations of disease are minutely analyzed and discussed in terms of those anatomical, biochemical, psychological, physiological, and pathological factors which have a role in their production. Highly interesting chapters are devoted to such topics as fatigue, pruritus, sore tongue, and joint pain.

In addition "every effort has been made by thorough revision to improve and bring up to date all chapters in the book. New chapters have been added on: Growth and Sex Development, Generalized Vasospasm and Arterial Hypertension, and Lymphadenopathy and Diseases of the Lymphatic System." Each chapter begins with a list of topics to be discussed and concludes with a concise summary of its content. There are 191 illustrations with 6 color plates. The array of contributors, usually leading authorities in the various subjects discussed, is impressive.

The usual criticisms of any book with many contributors applies here, i.e., slight overlapping of material and some chapters more up to date than others. However, to any person interested in the pathophysiology of disease, and certainly to all medical students, this book is highly recommended.

M. LOTZ

THE PHYSIOLOGY OF FISHES. Volume I, Metabolism. Margaret E. Brown, Ed. New York, Academic Press Inc., 1957. xiii, 447 pp., illus. $12.00.

This volume will be welcomed by zoologists whose spectra of interest may range from the purely scientific areas of gross and microscopic anatomy, histochemistry, physiology, biochemistry, and instrumentation, to the realm of the fishery biologist. The work encompasses such areas as respiration, circulation, digestion, excretion, endocrinology, development and biochemical composition of fishes. The term, fishes, here includes the classes Agnatha, Chondricthys, and Osteichthys. These forms present an almost overwhelming and provocative array of anatomical, developmental, and physiological specialization which offer the experimental zoologist a host of unique opportunities. Even a casual perusal of the volume will prove most stimulating in this regard.

Each of the ten review chapters is succinctly written and a marvel of brevity. The latter is possibly the reason for the omissions and errors occasionally present and obvious to the careful reader. Unfortunately, the rather long interval between submission of the review manuscripts and their publication, as indicated by the paucity of bibliographical material during 1955, has caused those reviews encompassing the areas of most active research to become of historical interest. However, these are relatively few failings for a pioneer work. The volume is enthusiastically welcomed and heartily endorsed. It is to be hoped that the series will be continued.

WALTER CHAVIN

MAN AS AN ANIMAL. By W. C. Osman Hill. Hutchinson University Library Biological Sciences. H. Munro Fox, Ed. London, Hutchinson Library, 1957. 176 pp. $1.50.

The place of man in the universe has been a perennial problem from time immemorial yet no satisfactory solution, generally accepted by man, has been reached. To simplify the problem somewhat, it may be said that there are currently two approaches. The first of these is the biological approach which holds that man is an animal, subject to all the laws which operate in the living world, and nothing else. Nothing else, at least, that can be subjected to the rigorous methods demanded by modern science. Adiametrically opposed approach holds that while man may be an animal, he definitely is something very much more than this. He possesses attributes not found in animals which have been described as "mind, or soul, or spirit." This latter position holds that man is a special creation; in the universe but not of it. That his activities are subject to laws which transcend the material aspect of his body and which are, therefore, of a transcendental nature. These laws, then, are largely to be understood through hope and faith so are not scientifically demonstrable.

Virtually all biologists maintain the first position, whereas most of our social, educational, and religious attitudes stem from the second position. In this very readable small book, Dr. Hill has reviewed the biological evidence. It demonstrates quite unequivocably that man certainly is an animal. The evidence is derived largely from the long story of evolution, supplemented by developmental studies. Unlike many surveys of this sort, Dr. Hill has included brief summaries of the importance of the function and the structure in terms of physiology, the supposedly important part played by the endocrines, and the necessarily brief discussion of comparative psychology. Needless to say, genetics is not omitted and its contribution is duly noted.

This is a readable book. It covers an enormous territory, much of which is familiar to the biologist, but a great deal of it is undoubtedly new to the educated layman. For the biologist, this is a good review of the subject; for the educated layman, there are many difficult spots. The terminology of any specialized science is apt to be strange and confusing and, as always, this tends to obscure the central story. Nevertheless, there is a great deal

of information in the book not readily available in one place to students of man. Strangely enough, there is little or no reference to the part which the structure and function of the nervous system plays in the one thing which separates man from the animals, namely, human behavior. The complexities of the nervous system are just as much a product of the phylogeny and ontogeny of the human race as the characteristics of hair, color, teeth, bones, etc. Man is what he is because of all of these properties, but he is something more than these because the incredible complexity of the nervous system endows him with qualitative attributes not to be found in other animals.

This is a useful and readable book providing one does not stop his consideration with the purely biological approach.

H. S. BURR

TRANSPORT AND ACCUMULATION IN BIOLOGICAL SYSTEMS. By E. J. Harris. New York, Academic Press, 1956. vi, 291 pp. $7.80.

The foreword of this book states the author's aims: "This volume represents the result of an attempt to gather together some of the extensive amount of information which has been published more recently on the subjects of cellular inorganic chemistry and the kinetics of movement of inorganic and simple organic substances between cells and their surroundings." In this aim the author has succeeded remarkably well as is evident by the almost 700 references which are cited. The breadth of Dr. Harris' knowledge provides the strong point of this work.

The strong point, however, also exposes the weak point; in attaining such broad coverage it has been necessary to present a rather superficial view of many topics. The problems of transport in biology are very profound and challenge our fundamental understanding of many aspects of physical chemistry. It is somewhat disconcerting to read through a catalogue of these problems at the rate of a page per problem. For example, on page 38 in discussing osmosis Dr. Harris states, "In the absence of hydrostatic pressure difference there is no force acting on the liquid as a whole to produce a net flow." The work of Ussing, Pappenheimer and others seriously challenges this statement and certainly deserves discussion.

It is sometimes difficult to determine the intended audience. For example, on page 17 there appears a simple discussion of osmosis characteristic of elementary physical chemistry books; this is followed on page 20 by a ten parameter equation which is called up without very much preparation or explanation. Throughout the book many of the formal mathematical developments assume that the reader has a thorough grounding in thermodynamics and diffusion theory. Such a reader is, however, likely to be somewhat annoyed by much of the more elementary material. The difficulty may be that Dr. Harris writes for physiologists whose knowledge of physical chemistry ranges from the naïve to the profound, thereby making it extremely difficult to set the level of sophistication.

While I cannot recommend this book for students, I feel that research workers in the field will welcome the comprehensive literature survey.

This book may be likened to a review article, only the author, not being limited by the 50-page upper limit on reviews, was able to present a more complete summary of the work he covered.

HAROLD J. NOROWITZ

THE STRUCTURE OF NUCLEIC ACIDS AND THEIR ROLE IN PROTEIN SYNTHESIS. Biochemical Society Symposia, Number 14. E. M. Cook, Ed. New York, Cambridge University Press. 1957. 74 pp. $3.75.

This is the fourteenth of a series of Biochemical Society Symposia published annually. There has been an unfortunate delay in publication since the Symposium was held on February 18, 1956 and the book was not published until May 22, 1957.

A brief historical introduction to the morning session by N. W. Pirie touches on a variety of subjects from the unfounded assumptions made in nucleic acid research to the relationship between terminology and biological entity. R. Markham gives a very good and concise review of the structure of nucleic acids, and indicates the improbability of chain branching in RNA although more flexible views on this topic have also been published. M. H. F. Wilkins presents experimental data concerning the structure of DNA which are in accord with the Watson and Crick model, and discusses in an interesting manner the types of structural combinations of DNA with protamine and histone.

The afternoon session is introduced by J. N. Davidson who gives an excellent thumbnail sketch of the cytological aspects of the interrelationship of nucleic acids and protein synthesis. He mentions the increasing volume of evidence that RNA is involved in protein synthesis while DNA may be involved in the synthesis of certain specific proteins.

B. A. Askonas, J. L. Simkin, and T. S. Work present a survey of the literature bearing on the interrelationship between nucleic acid and protein synthesis. They point out the difficulties inherent in accepting nucleic acids as templates in the synthesis of proteins and suggest that patternization could be controlled through the influence of small nucleotides on reaction rates. E. F. Gale reviews the literature on amino acid incorporation by subcellular preparations from different sources with particular emphasis on the work from his laboratory. Gale has published a similar but more extensive review in the Harvey Lectures of 1956.

K. Burton presents a very comprehensive review of the topic in relation to bacteriophage multiplication. In this relatively simple biological system, in terms of participating components, the connection between DNA and protein synthesis can be shown more clearly.

This book brings to the foreground the facts as well as the hypotheses current in this field. Although there is appreciable evidence for a relationship between nucleic acids and protein synthesis, it may be that this does not present a cause-effect relationship but rather a co-occurrence of biological phenomena which are not directly related. For instance, it appears to be a fact that bacteria require a complete amino acid mixture for bacteriophage-DNA synthesis, but the interpretation, rather than being

one of a template synthesis, may well be that a limited number of enzymes necessary for the synthesis of the DNA have to be synthesized. Once adequate amounts of these enzymes have been synthesized, DNA synthesis may then proceed without any further protein synthesis. (This amount of protein synthesis may represent a small percentage of the total protein synthesis and may not be completely inhibited by chloramphenicol.)

Regardless of the interpretation of the experimental results, this book presents an excellent summary of the pertinent literature as well as uninhibited discussions by competent investigators of the type which usually do not find their way into print. The book is highly recommended, not only to investigators directly interested in the field, but also to more generally oriented graduate students and biologists who wish to be familiar with recent developments in a rapidly advancing field.

E. S. CANELLAKIS

THE MICROBIAL WORLD. By Roger Y. Stanier, Michael Doudoroff, and Edward A. Adelberg. Englewood Cliffs, New Jersey, Prentice-Hall, Inc., 1957. xxii, 682 pp. $8.00.

In the preface to this new text on microbiology the authors declare: "Microbiology was for a long time very largely an applied science, empirical in outlook, and isolated from the mainstreams of biological thought." With but few exceptions, the textbook literature of microbiology amply testifies to the accuracy of this statement. The applied specialties have not only dominated textbook writing in number but in content. The classical introductory volume in microbiology consists of a few perfunctory chapters on microbial taxonomy and physiology, an arithmetical summary of the population dynamics of *Escherichia coli* in the test tube, followed by chapters on the microorganisms that fix nitrogen, cause disease, ferment sugar to alcohol, lurk in milk and water, and, perhaps, put holes in cheese. The recent rebellion against this form has tended, unfortunately, to exaggerate the molecular approach, resulting in attempts to condense a generous portion of physical and biological chemistry into a microbiology text.

It is against this background that *The Microbial World* stands out so brilliantly. This work is a clearly written and well-illustrated introduction to the *biology* of microorganisms. The first and major portion of the book establishes the range and diversity of microorganisms, proceeds with an extended account of their anatomy and physiology, their myriad biochemical activities, and concludes with what is probably one of the few stimulating discussions available of the problems of microbial classification. Stressed constantly by the authors is the profit derived from the application of genetics and evolution to microbial problems, and, in turn, the contribution made to these universal biological themes by studies in microbiology. With this fund of information now made available to the reader, part two of the book explores the mutual relations between microorganisms and their environment in a far more satisfactory manner than the mere compilation of what microorganisms do and do not do.

It is pleasant to recommend this stimulating and exciting book to all those who wish an introduction to or a renewed acquaintance with the microbial world.

STANLEY E. MILLS

ANESTHESIOLOGY AND RELATED PROBLEMS. C. R. Stephen, Ed. The Annals of the New York Academy of Sciences, vol. 66, art. 4, pp. 841-1022. New York, 1957. $4.00.

In May, 1956 a conference was held at the New York Academy of Sciences on the subject "Anesthesia and Related Problems." The results of this conference are now presented in a series of 15 papers. On the whole these papers are characterized by an academic and objective approach to many of the clinical problems relating to anesthesia but because of their fundamental nature some of them can also be highly recommended to all those interested in applied respiratory physiology and acid-base balance. The careful analysis of W. A. Spencer of some of the variables affecting pulmonary ventilation is especially interesting, being supported as it is by careful measurements of respiration under various normal and abnormal conditions in the human. J. V. Maloney and J. L. Whittenberger introduce an important concept in the understanding of the mechanical factors involved in the physiological effects of positive pressure respiration during their discussion of pulmonary circulation in the presence of positive pressure, while Roberts and her associates from Cornell provide us with a complete and up-to-date summary on the acid-base changes accompanying respiratory alkalosis. Roberts' data, while suffering somewhat from an unfortunate transposition of illustrations, describe in detail the metabolic responses to the various stages of respiratory alkalosis and propose a method for making the difficult differential diagnosis between severe end-stage respiratory alkalosis and metabolic acidosis. Articles such as these three make this symposium valuable for the advanced student, while with few exceptions the remaining articles consist of fundamental reviews that will prove of value to the nonspecialist.

NICHOLAS M. GREENE

MERCURY AND ITS COMPOUNDS. By Cecil V. King, Harry Gold, Harold Wolff, *et al.* The Annals of the New York Academy of Sciences, vol. 65, art. 5, pp. 357-652. New York, 1957. $3.50.

This is the annotated transcript of a symposium held by the New York Academy of Sciences on April 6 and 7, 1956 for the interesting purpose of discussing recent advances in the physics and chemistry of mercury, the pharmacology and toxicology of inorganic mercury and the organomercurial compounds, and the application of the latter to clinical medicine. It is at once apparent that these subjects are more unified in name than in practice; a clinician-reader might muse sadly over the paucity of references in Part III (Clinical Medicine) in comparison with Part I (Physics and Chemistry) unless he were intimidated by titles like "The Growth of

Mercury Crystals from the Vapor" or "Oxymercuration of Alkenes." There is a good summary of "Relationship between Clinical Structure and Biological Activity in Mercurial Compounds," by Harris L. Friedman, a brief section, with references, on "Toxicology of Inorganic Mercury" by Leonard J. Goldwater, and an excellent short discussion by Alvin Farah of histochemical techniques for localizing the site of action of mercurial diuretics.

The section on Clinical Medicine, with the exception of a paper by Raymond Weston summarizing work done by the Montefiore group on the mechanism of mercurial diuresis and a short, informative discussion of "Bioassay of Organomercurials" by Theodore Greiner, is overloaded with dogmatic statements and (in 1957) a pedestrian series of successfully treated cases reflecting the general clinical acceptance of oral and parenteral mercurial diuretics. There is little critical or factual discussion of one of the nagging problems the clinician faces: when is mercury helpful and when toxic in patients with edema and renal disease? On page 621 one reads: "We follow the generally accepted view that there are few contraindications to the use of mercurials in patients with congestive heart failure, regardless of the state of the kidneys." Three pages later, on page 624, another authority thinks that ". . . the most important contraindications to oral mercurial diuretic therapy are uremia and the presence of significant primary renal disease." Interspersed with this sort of comment there is, to be sure, some useful and interesting clinical information.

Most of the papers are carefully documented with bibliographical references which will add to the value of this collection for clinicians and pharamacologists interested in mercury.

FRANKLIN H. EPSTEIN

HUMAN BLOOD GROUPS AND INHERITANCE. By S. D. Lawlor and L. J. Lawlor. Cambridge, Harvard University Press, 103 pp. $1.50.

In the introduction it is stated that this monograph was designed for the biology student and the interested lay reader. The first chapter, which deals with an historical survey of blood groups, might be of interest to the lay reader, but thereafter the material is strictly for the biology student. An extensive consideration of human blood groups and their inheritance along with brief considerations of blood grouping and blood banking techniques are contained in this monograph. These usually are rather difficult subjects to present clearly, and the authors of this book appear to have done an excellent job. The book does not read as a novel, and in order to obtain an understanding of immunohematology one must concentrate on each line, but the material is presented in a clear manner and in a good sequential fashion.

Primary emphasis in this book is placed on the inheritance of the common and uncommon blood groups. In this respect the information presented is very suitable for the practising physician. On the other hand, its greatest weakness is in the actual hematologic techniques which are described. They are so superficial that they probably should be deleted

but, fortunately, they constitute only a very small portion of the over-all presentation. Another excellent feature of this book is the glossary of immunologic and genetic terms. Along with a very complete index, this inexpensive little monograph should serve as an excellent up-to-date reference for problems relating to the inheritance of blood groups in man.

STUART C. FINCH

HISTORY OF NURSING SOURCE BOOK. By Anne L. Austin. New York, G. P. Putnam's Sons, 1957. 480 pp. $7.50.

This volume will be an invaluable addition to the libraries of schools of nursing. Designed to be used as a supplement to the usual history of nursing textbooks, it includes, as the author states in the preface, "excerpts from the most significant writings comprising the history of nursing from the era before the birth of Christ up to the founding of the first professional school of nursing in America in 1873." Miss Austin, who for many years taught the history of nursing at the Frances Payne Bolton School of Nursing of Western Reserve University, not only recognized the need for such a source book but has met the need in exemplary fashion. Making use of primary and secondary sources, the book is the result of painstaking research and in turn will open up avenues of research for many students of nursing.

The sources are arranged chronologically, starting with quotations from the Old Testament, the Talmud, and ancient Sanskrit texts. References to nursing in the Middle Ages and in the nineteenth century are selected from less accessible materials than those usually found in textbooks. The final chapter contains selected documents of early war nursing in the United States.

The methods of documentation and indexing are those of sound historical research and as such set a pattern to be followed by students working in the area of nursing research. The material should also be of interest to social scientists. We are indebted not only to the author but to the editor and publishers for this volume, the latest in the Modern Nursing Series.

ELIZABETH S. BIXLER

ALBERT SCHWEITZER, THE STORY OF HIS LIFE. By Jean Pierhal. New York, Philosophical Library, 1957. 160 pp. $3.00.

This is apparently an abridged version of *Albert Schweitzer: Das Leben eines guten Menschen* by Jean Pierhal, "describing Schweitzer's childhood experiences, his researches and travels, his suffering and dedication." In the 160 brief pages of this work the fascinating drama of the great doctor's life is told simply, clearly, and with admirable objectivity while successfully conveying the character and the magnitude of his genius.

There are 22 black and white photographic illustrations including one of *The Sorrowful Negro* of the Colmar statue by Bartholdi which made such a deep impression on Schweitzer in his boyhood.

It might be expected that so great a man as Schweitzer, who has contributed equally to philosophy, theology, music, sociology, and medicine, would deserve something more in the way of biography than a mere 160 pages. For example, it would be very interesting to know more of his doctorate of medicine dissertation, submitted to the University of Strasbourg, which was concerned with the interpretation of Jesus as a psychiatrist. Similarly, there is a rather cursory treatment of his dissertation for his doctorate of philosophy in which he had made the discovery that Kant had woven into his famous *Critique of Pure Reason* an earlier study in the philosophy of religion.

It would appear that this work is especially suited as an introductory text to those who have only a passing acquaintance with this man, in particular younger people who undoubtedly would find sustaining inspiration in the pattern of life of this already legendary figure. To those who are already well acquainted with Schweitzer this book can serve as a convenient outline and summary from which one can embark on a more intensive study of the original works.

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